BOVINE SOMATOTROPIN SUPPLEMENTATION DURING THE TRANSITION PERIOD AND EARLY LACTATION AS A MANAGEMENT STRATEGY TO IMPROVE PHYSIOLOGICAL ADAPTATIONS, LIVER FUNCTION, HEALTH AND PRODUCTIVITY OF DAIRY COWS

By

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by

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To Kellym, with love.

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TABLE OF CONTENTS

page

ACKNOWLEDGMENTS iv

LIST OF TABLES ix

LIST OF FIGURES xiii

ABSTRACT xvi

CHAPTER

1 INTRODUCTION 1

2 LITERATURE REVIEW 7

- Dry Matter (Feed) Intake during the Transition Period 7
- Changes in Body Weight and Body Condition Score during the Transition Period .. 12
- Hormonal and Metabolic Adaptations during the Transition Period 18
- Liver Function during the Transition Period 28
- Mammary Gland Function during the Transition Period 36
- Diseases and the Transition Period 42
- Recombinant Bovine Somatotropin (bST) and Lactation in Dairy Cows 49
- Strategies to Improve Metabolic Adaptations during the Transition Period 55

3 MATERIALS AND METHODS 63

- Animals 63
- Treatments 64
  - Experimental Design 64
  - Feeding 65
- Body Weight and Body Condition Score 65
- Colostrum and Milk Samples 65
- Blood Collection, Handling and Storage 67
- Liver Biopsies, Handling and Storage 68
- Radioimunoassays 69
  - Second Antibody Preparation 69
  - Insulin, Somatotropin and IGF-I Iodination and Protein Separation 70
- Insulin Assay 72

Somatotropin Assay 74
IGF-I Assay 76
    Extraction of IGF-I from binding proteins 76
    Assay 77
Calcium Assay 78
Glucose Assay 80
Non-esterfied Fatty Acids (NEFA) Assay 81
β-Hydroxybutyrate (B-HBA) Assay 83
Total Lipid Determination of Liver Samples 84
Triacylglycerol (TAG) Determination of Liver Samples 85
Messenger Ribonucleic Acid (mRNA) Determination by DOT BLOT Procedure ... 87
    Extraction of mRNA 87
    Dot Blot Membrane Transfer 88
    Hybridization 89
    Incubation, Film Development and Membrane Stripping 92
Calving Variables, Health Status and Reproductive Performance 92
Statistical Analyses 95
    Productive Responses, Physiological Changes, Calving Information and Reproductive Performance 95
    Health information and reproductive performance 100

4 RESULTS 102

Physiological Adaptations during the Prepartum Period 102
    Body Weight and Body Condition Score 102
    Hormones and Growth Factor 103
    Calcium and Metabolites 109
Physiological Adaptations during the Postpartum Period 116
    Body Weight and Body Condition Score 116
    Hormones and Growth Factor 126
    Calcium and Metabolites 143
Liver Metabolism of Lipids and Carbohydrates 145
Calving Variables 155
Health Information and Reproductive Performance 167
Milk Yield and Milk Composition 171

5 DISCUSSION 187

APPENDIX

A RESIDUAL CORRELATION COEFFICIENTS AMONG HORMONES AND METABOLITES MEASURED FROM 21 D PREPARTUM THROUGH CALVING IN PLASMA OF HOLSTEIN COWS 244

B RESIDUAL CORRELATION COEFFICIENTS AMONG HORMONES AND METABOLITES MEASURED DURING THE EARLY POSTPARTUM PERIOD (0-63 DIM) IN PLASMA OF HOLSTEIN COWS 245

C SUMMARY OF HETEROGENEITY OF REGRESSION FOR VARIABLES MEASURED DURING EXPERIMENT 246

D RESIDUAL CORRELATION COEFFICIENTS AMONG LIVER MEASURES DURING THE TRANSITION PERIOD OF HOLSTEIN COWS 247

E SUMMARY OF TREATMENT EFFECTS OF MULTIPAROUS HOLSTEIN COWS SUPPLEMENTED WITH bST DURING PREPARTUM OR DURING POSTPARTUM PERIODS COMPARED TO NON-SUPPLEMENTED CONTROLS 248

F SUMMARY OF TREATMENT EFFECTS OF MULTIPAROUS HOLSTEIN COWS SUPPLEMENTED WITH bST DURING PREPARTUM AND POSTPARTUM PERIODS COMPARED TO NON-SUPPLEMENTED CONTROLS 249

LIST OF REFERENCES 250

BIOGRAPHICAL SKETCH 269

LIST OF TABLES

Table Page

1. Dry matter concentrations and chemical composition of anionic close-up diet (CUD) and fresh-cow TMR fed to Holstein Cows 66

2. Arrangement of tubes for radioimmunoassay of insulin 73

3. Arrangement of tubes for radioimmunoassay of ST 76

4. Arrangement of tubes for radioimmunoassay of IGF-I 78

5. Standards for determination of calcium by flame atomic absorption spectometry.....79

6. Standard dilutions used in the glucose assay 80

7. Arrangement of tubes and solutions used to make plasma protein-free filtrates 81

8. Standard dilutions for NEFA determination 82

9. Standard dilutions used for determination of TAG. 86

10. Least squares analyses of variance for body weight (BW) and body condition score (BCS) of Holstein cows during the prepartum period 104

11. Least squares means and SEM for body weight (BW) and body condition score (BCS) of Holstein cows for treatments and season of calving during the prepartum period 105

12. Least squares analyses of variance for concentrations of insulin, somatotropin and insulin-like growth factor-I (IGF-I) in plasma of Holstein cows during the prepartum period 110

13. Least squares means and SEM for concentrations of insulin(INS), somatotropin (ST) and insulin-like growth factor-I (IGF-I) for treatments and season of calving in plasma of Holstein cows during the prepartum period 111

14. Least squares analyses of variance for concentrations of calcium and glucose in plasma of Holstein cows during the prepartum period 117

15. Least squares analyses of variance for concentrations of non-esterified fatty acids and β-hydroxybutyrate in plasma of Holstein cows during the prepartum period.... 118

16. Least squares means and SEM for concentrations of calcium (Ca), glucose (GLC), non-esterified fatty acids (NEFA) and β-hydroxybutyrate (B-HBA) for treatments and season of calving during the prepartum period 119

17. Least squares analyses of variance for body weight (BW) and body condition score (BCS) of Holstein cows from 0-63 DIM 127

18. Least squares analyses of variance for body weight (BW) and body condition score (BCS) of Holstein cows from 64-150 DIM 128

19. Least squares analyses of variance for body weight (BW) and body condition score (BCS) of Holstein cows from 0-150 DIM 129

20. Least squares means and SEM for body weight (BW) and body condition score (BCS) of Holstein cows for treatments and season of calving during various time periods postpartum 130

21. Cubic regression coefficients that describe treatment effects on body weight (BW) of Holstein cows from 0-150 DIM 131

22. Cubic regression coefficients that describe treatment effects on body condition score (BCS) of Holstein cows from 0-150 DIM 133

23. Least squares analyses of variance for concentrations of insulin, somatotropin and insulin-like growth factor-I (IGF-I) in plasma of Holstein cows during 0-63 DIM 137

24. Least squares means and SEM for concentrations of insulin (INS), somatotropin (ST) and insulin-like growth factor-I (IGF-I) in plasma of Holstein cows for treatments and season of calving during 0-63 DIM 138

25. Cubic regression coefficients that describe treatment effects on concentration of somatotropin in plasma of Holstein cows from 0-63 DIM 139

26. Cubic regression coefficients that describe treatment effects on concentrations of IGF-I in plasma of Holstein cows from 0-63 DIM 141

27. Least squares analyses of variance for concentrations of glucose and calcium in plasma of Holstein cows during postpartum period... 146

28. Least squares analyses of variance for concentrations of non-esterified fatty acids and β-hydroxybutyrate in plasma of Holstein cows during postpartum period........ 147

29. Least squares means and SEM for concentrations of glucose (GLC), calcium (Ca), non-esterified fatty acids (NEFA) and β-hydroxybutyrate (B-HBA) for treatments and season of calving during the postpartum period ... 148

30. Cubic regression coefficients that describe treatment effects on concentrations of β-hydroxybutyrate (B-HBA) in plasma of Holstein cows from 0-28 DIM ... 149

31. Least squares analyses of variance for percentages of fat (LFAT) and triacylglycerol (TAG) in liver of Holstein cows (wet weight basis) during prepartum and postpartum periods ... 156

32. Least squares analyses of variance for mRNA expression in liver of Holstein cows for *pyruvate carboxylase* (PC), *phosphoenolpyruvate carboxykinase* (PEPCK) and *microsomal triacylglycerol transfer protein-1* (MTP) ... 157

33. Least squares means and SEM for percentages of fat (LFAT) and triacylglycerol (TAG) in liver of Holstein cows on a wet weight basis, and for liver mRNA expression of *pyruvate carboxylase* (PC), *phosphoenolpyruvate carboxykinase* (PEPCK) and *microsomal triacylglycerol transfer protein-1* (MTP) for treatments and day from 21 d prepartum to +28 DIM ... 158

34. Least squares means and SEM for percentages of fat (LFAT) and triacylglycerol (TAG) in liver of Holstein cows on a wet weight basis for each day of biopsy ... 159

35. Least squares means and SEM for mRNA expression in liver of Holstein cows for *pyruvate carboxylase* (PC) and *phosphoenolpyruvate carboxykinase* (PEPCK) for each day of biopsy ... 160

36. Least squares means and SEM for mRNA expression in liver of Holstein cows for *microsomal triacylglycerol transfer protein 1* (MTP) for each day of biopsy ... 161

37. Least squares analyses of variance for colostrum immunoglobulin content, calf birth weight and calving difficulty of Holstein cows ... 168

38. Least squares means and SEM for colostrum immunoglobulin content (Colost), calf birth weight (CLFWT) and calving difficulty (DCV) of Holstein cows for treatment groups and season of calving ... 169

39. Disease incidence and reproductive performance of multiparous Holstein cows supplemented or not with bST ... 172

40. Summary of logistic regression analyses of the associations between TRT, SEA and diseases, and variables of reproductive performance of multiparous Holstein cows during 0-60 DIM ... 173

41. Least squares analyses of variance for milk yield of Holstein cows during 3-63 and 64-150 DIM ... 178

42. Least squares analyses of variance for milk yield of Holstein cows during 3-150 DIM and for Actual 305-d MY 179

43. Least squares means and SEM for milk yield (MY) of Holstein cows for treatments and season of calving 180

44. Cubic regression coefficients that describe treatment effects on milk yield of Holstein cows during 3-150 DIM........ 182

45. Least squares analyses of variance for percentages of milk fat and milk protein and somatic cell count (SCC) in milk of Holstein cows from 3-63 DIM 185

46. Least squares means and SEM for percentages of milk fat (FAT) and milk protein (PROT), and somatic cell count in milk (SCC) of Holstein for cows treatments and season of calving 186

LIST OF FIGURES

Figure Page

1. Some metabolic pathways of adipocytes and their adaptations during transition period 21

2. Schematic drawing of rat liver tissue 29

3. Relationships among metabolic disorders 48

4. Backbone structure of the hGH×(hGHbp)2 complex 50

5. Least squares means depicting body weights (BW) of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. Vertical dashed line indicates calving. Shaded area represents time during which full dose of bST was supplemented to all cows 106

6. Least squares means depicting body condition scores (BCS) of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. Vertical line indicates calving. Shaded area represents time during which full dose of bST was supplemented to all cows 107

7. Least squares means depicting insulin concentrations in plasma of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. Vertical dashed line indicates calving. Shaded area represents period after calving 112

8. Least squares means depicting somatotropin concentrations in plasma of multiparous Holstein cows supplemented or not with bST during the prepartum and/or the postpartum periods. Vertical dashed line indicates calving. Shaded area represents period after calving 113

9. Least squares means depicting IGF-I concentrations in plasma of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. Vertical dashed line indicates calving. Shaded area represents period after calving 114

10. Least squares means depicting calcium concentrations in plasma of multiparous Holstein cows supplemented or not with bST during the prepartum and/or during the postpartum periods. Vertical dashed line indicates calving. Shaded area represents period after calving 120

11. Least squares means depicting glucose concentrations in plasma of multiparous Holstein cows supplemented or not with bST during the prepartum and/or during the postpartum periods. Vertical dashed line indicates calving. Shaded area represents period after calving. 121

12. Least squares means depicting NEFA concentrations in plasma of multiparous Holstein cows supplemented or not with bST during the prepartum and/or during the postpartum periods. Vertical dashed line indicates calving. Shaded area represents period after calving. 122

13. Least square means depicting β-hydroxybutyrate (B-HBA) concentrations in plasma of multiparous Holstein cows supplemented or not with bST during the prepartum and/or during the postpartum periods. Vertical dash line indicates calving. Shaded area represents period after calving. 123

14. Cubic regression curves depicting body weight changes of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. Model: bST, season, bST*season effect, using cow(bST*season) as error, with wk to the third order as covariate. Shaded area represents time during which full dose of bST was supplemented to all cows. 132

15. Cubic regression curves depicting body condition score of multiparous Holstein cows supplementedor not with bST during the prepartum and/or postpartum periods. Model: bST, season, bST*season effect, using cow(bST*season) as error, with wk to the third order as covariate. Shaded area represents time during which full dose of bST was supplemented to all cows. 134

16. Cubic regression curves depicting somatotropin concentrations in plasma of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. bST, season, bST*season effect, using cow(bST*season) as error, with day to the third order as covariate. 140

17. Cubic regression curves depicting IGF-I concentrations in plasma of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. Model: bST, season, bST*season effect, using cow(bST*season) as error, with day to the third order as covariate. 142

18. Cubic regression curves depicting β-hydroxybutyrate (B-HBA) concentrations in plasma of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. Model: bST, season, bST*season using cow(bST*season) as error, with day to the third order as covariate 150

19. Least squares means depicting percentages of total fat in liver (LFAT, wet weight basis) of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. ^{a,b} Superscripts within day from calving indicate means that differed at P<0.05. 162

20. Least squares means depicting percentage of triacylglycerol in liver (TAG, wet weight basis) of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. ^{a,b} Superscripts within day from calving indicate means that differed at P<0.05. 163

21. Least squares means depicting mRNA expression of *pyruvate carboxylase* (PC) of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. ^{a,b} Supercripts within day from calving indicate means that differed at P<0.05. 164

22. Least squares means depicting mRNA expression of *phosphoenolpyruvate carboxykinase* (PEPCK) of multiparous Holstein cows supplemented or not with bST during the prepartum and/or periods. ^{a,b} Superscripts within day from calving indicate means that differed at P<0.05. 165

23. Least squares means depicting mRNA expression of *microsomal triacylglycerol transfer protein 1* (MTP) of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. ^{a,b} Superscripts within day from calving indicate means that differed at P<0.05. 166

24. Least squares means depicting daily milk yield of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. Shaded area represents time during which full dose of bST was supplemented to all cows 181

25. Cubic regression curves depicting mean daily milk yield of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum period. Model: bST, season, and the two-way interaction of bST*season effect, using cow nested with bST*season as error, with day to the third order as covariate. Shaded area represents time during which full dose of bST was supplemented to all cows 183

26. Cubic regression curves depicting mean daily milk yield (MY) of multiparous Holstein cows supplemented or not with bST (500 mg/14 d) from 64-150 DIM. Model: bST, season, bST*season effect, using cow(bST*season) as error, with day to the third order as covariate. 184

Abstract of Dissertation Presented to the Graduate School
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BOVINE SOMATOTROPIN SUPPLEMENTATION DURING THE TRANSITION PERIOD AND EARLY LACTATION AS A MANAGEMENT STRATEGY TO IMPROVE PHYSIOLOGICAL ADAPTATIONS, LIVER FUNCTION, HEALTH AND PRODUCTIVITY OF DAIRY COWS

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Objectives were to evaluate effects of supplemental bST (0.4 mL/14d, 10.2 mg/d, POSILAC®) during prepartum and/or postpartum periods and early lactation on physiological adaptations [changes in body weight (BW), body condition score (BCS) and concentrations of insulin, somatotropin, IGF-I, calcium, glucose, non-esterified fatty acids and β-hydroxybutyrate in plasma], on metabolism of lipids and carbohydrates in liver (fat deposition and gene expression of key enzymes), on health, and on milk yield (MY) and composition. Multiparous Holstein cows were assigned randomly to a 2x2 factorial arrangement of treatments (TRT) to give four groups (I=no bST, n=26; II=bST postpartum, n=25; III=bST prepartum, n=27; IV= bST prepartum and postpartum, n=25). Biweekly supplementation of bST began 21 d before expected calving through 63 d in milk (DIM). From 64 DIM through the end of lactation, all cows were supplemented biweekly with a full bST dose (1.2 mL/14d, 35.7 mg/d). Blood samples were collected from all cows thrice weekly, and liver biopsies were taken from ~9 cows per TRT group at ~-21, +2, +14 and +28 d from calving. Cows were fed an anionic diet prepartum and a cationic diet during the postpartum period for ad libitum consumption. Supplementing bST only prepartum (III) did not impact physiological adaptations, but in TRT II cows (bST Post only), supplementation increased concentrations of β-hydroxybutyrate in plasma and accumulation of triacylglycerol in liver during the first 28 DIM. Milk production, incidence of diseases and reproductive performance of TRT II cows were similar to cows on TRT I and III (control and bST Prepartum). For cows on TRT IV (bST Prepartum and Postpartum), milk production was increased by 12.8% (4.64 kg) during the first 150 DIM, and milk production was higher until the end of lactation, and the cows were healthier than controls or cohorts. Compared to controls, cows on TRT IV had similar BW and BCS changes, showed increased concentrations of IGF-I in plasma during experiment, similar concentrations of hormones and metabolites in plasma, and improved liver function. These observations indicate that supplementation of low doses of bST improves metabolic adaptations and health of dairy cows, which is reflected in increased milk production.

CHAPTER I
INTRODUCTION

For dairy cows, the period around parturition has been considered the most critical phase during the whole lactation cycle (Chilliard, 1999). During this phase, there are physiological priorities that require metabolic adaptations of many tissues and organs to allow cows to meet general maintenance of body and their productive needs (Bauman and Elliot, 1983). These adaptations provide adequate nourishment to the fetus during its final phase of gestational development (Bell, 1995), allow the initiation of lactogenesis and parturition (Head, 2000), and thereafter copious milk production (Capuco et al., 1997).

Late pregnancy imposes a substantial cost to the dairy cow. The total requirements for nutrients at the end of pregnancy are about 75% greater than in nonpregnant animals of the same body weight (Bauman and Currie, 1980). At 250 d of pregnancy, a 35 kg bovine fetus requires daily a total of 2,335 Kcal of energy, and about 220 g of metabolizable amino acids (AA) for its growth. In addition, the growth of the uteroplacental tissues (placentomes, endometrium, myometrium) also imposes an extra requirement for nutrients. It has been estimated that a gravid uterus (64 kg at 250 d of pregnancy) utilizes 46, 72 and 12% of the total maternal supply of glucose, amino acids and acetate, a substantial demand for nutrients during late pregnancy (Bell, 1995).

In addition to development of uterine tissues and the fetus, there is a marked increase in mammary gland activity as parturition approaches, a period termed lactogenesis. Lactogenesis has been divided into two phases (I and II), and in dairy cows it begins about 30-14 d before calving. Phase I is characterized by a renewal of damaged and senescent mammary epithelial cells in the gland (Capuco et al., 1997), by morphological and biochemical changes in these cells, and by the synthesis of a precolostral fluid (secretion composed of proteins, lipids and carbohydrates). Phase II begins immediately before parturition and is characterized by copious secretion of colostrum (colostrogenesis; Oliver and Sordillo, 1989). For the latter process to occur, there must be an increase in uptake of nutrients such as glucose, amino acids and nonesterified fatty acids (NEFA) by the mammary gland to synthesize lactose (Holt, 1983), milk proteins (Boisgard et al., 2001) and milk lipids (Shennan and Peaker, 2001). This is accomplished by the increased flow of blood to the gland, that has been observed in both small and large ruminants. In dairy goats, beginning at d 2 prepartum and continuing through d 1 and 0.5 prepartum, there was a 400% increase in blood flow to the gland and 150% increase in uptake of glucose when compared to levels observed during d 3 prepartum (Davis et al., 1979). In dairy cows, it was observed that the increase in blood flow began at d 2 prepartum (Malven et al., 1987). The galactopoietic activity of the mammary gland imposes a significant demand to the dairy cow during this very critical phase. For example, the production of just 10 kg of colostrum on the day of calving requires an extra 11 Mcal of energy, 140 g of protein, 23 g of calcium, 9 g of phosphorus and 1 g of magnesium that must be obtained from the diet or by mobilization of body stores (Goff and Horst, 1997).

After calving (following the onset of lactation) the function of the mammary gland is the physiological priority for the dairy cow. The gland has a massive requirement for glucose to produce lactose and provide energy, and also for other nutrients that are used to synthesize other milk components. Estimates are that at d 4 of lactation, a dairy cow producing 30 kg milk/d has a mammary uptake of 85 and 75% of the daily predicted postabsorptive glucose and amino acid supply (Bell et al., 1995). These data agreeds with earlier estimates reported by Bickerstaffe and Annison (1974); they also estimated that the mammary uptake of glucose during lactation could reach about 85% of the total available sources.

Because of the increased and very substantial demand for nutrients during the peripartum period, the dairy cow faces great nutritional challenges as parturition approaches to meet these demands. During the prepartum period there is a decrease in feed intake, and during the early postpartum period, feed intake does not meet the requirements for milk production (Grummer, 1995). To obtain adequate quantities of nutrients for maintenance and milk production, the dairy cow must mobilize its body reserves to meet the demands (Drackley, 1999), a physiological process of adaptation termed *homeorhesis*. It occurs in conjunction with the maintenance of the physiological equilibrium, termed *homeostasis* (Bauman and Currie, 1980).

During periods of *homeorhesis* there is overall adaptation of the body to meet nutritional needs. Significant changes occur in the metabolism of body tissues responsible for storage of nutrients, and in organ(s) where the biochemical reactions responsible for synthesis of nutrients occurs (e.g., liver). Some of these changes include increased lipolysis and decreased lipogenesis in the adipose tissue, which leads to the release of NEFA (McNamara, 1991); increased hepatic gluconeogenesis and glycogenolysis (Greenfield et al., 2000) that leads to increased synthesis of glucose; decreased use of glucose and, in general, increased use of lipids as energy sources in all body tissues (Bauman and Currie, 1980); the mobilization of protein reserves from muscle and other body tissues, which gives rise to use of lactate and AA in the process of gluconeogenesis (Bauman and Elliot, 1983); and increased calcium absorption from the intestines and mobilization from bone tissue (Kronfeld, 1971).

These described adaptations are mediated by hormonal changes that occur to initiate parturition and lactogenesis (Grummer 1995). Insulin and glucagon have primary roles in the process of homeostatic regulation. Their principal function is to maintain the constancy of glucose supply. In addition to the homeostatic hormones, there are changes in concentrations of other hormones such as estrogen (E_2), progesterone (P_4), parathormone (PTH), vitamin D_3 (VIT D_3), prolactin (PRL), placental lactogen (PL) and somatotropin (ST; Tucker, 1985). Subsequent studies indicated that some of these hormones specifically target tissues involved in metabolic adaptations, and therefore they were considered to be homeorhetic hormones (Chilliard, 1999; Ingvartsen and Andersen, 2000).

The peripartum period is characterized by the occurrence of many commonly known metabolic disturbances/diseases of dairy cows (Drackley, 1999). Fatty liver, clinical and subclinical ketosis, and milk fever (MF) are just three of the disorders that are caused by imbalances in the metabolic adaptations described previously (Morrow, 1976; Goff and Horst, 1997; Heuer et al., 1999). In addition, these primary disorders also predispose the animals to other disorders such as metritis (MET), mastitis (MAST), displaced abomasum (DA) and poor reproductive performance (Gearhart et al., 1990;

Staples et al., 1990). As a consequence, they lead to poor productive performance during the lactation that follows parturition.

Due to the metabolic complexity, physiological priorities and changes in DMI observed during the periparturient period, some authors referred to this phase as the “transitional phase” (Gerloff, 1988) or “transition period” (Grummer, 1995). By the definition of both authors, this period begins at 3 wk prepartum and extends through calving. In addition to this prepartum period, Grummer (1995) also suggested a postpartum period that would begin right after parturition and last until 3 wk of lactation; thus the total period is 6 wk. Drackley (1999) also stressed and reinforced the importance of defining the periparturient phase as the transition period, and supported the length as defined by Grummer (1995), because of the greater occurrence of health problems that had their genesis during this relatively short period of time (Curtis et al., 1983; Thompson et al., 1983; Goff and Horst, 1997; Kelton et al., 1998).

One management strategy that has been considered to improve the metabolic adaptations during the transition period is supplementing exogenous bovine somatotropin (bST). The well-known effects of bST on feed intake, on metabolic and lactational hormones, and on the ability to increase the availability of glucose precursors in the lactating cow may be an important way to bring about desired effects during the transition period (Gluckman and Breier, 1987; Pell and Bauman, 1987; Bauman and Vernon, 1993; Eppard et al., 1996). It is known from previous studies (Stanisiewski et al., 1992; Garcia-Gavidia et al., 2000, Gulay et al., 2003b) that supplementing dairy cows with low doses of bST around parturition (prepartum and postpartum injections) had small but positive effects on metabolism, hormones and milk production.

Garcia-Gavidia et al. (1998) showed that supplementation of cows with low doses of bST during the prepartum and postpartum periods tended to allow them to better maintain their feed intake during the prepartum period and they also had more rapid increase in feed intake postpartum. Although changes observed were not significant, bST-supplemented cows produced more milk during the first 65 d postpartum, and these supplemented cows also showed changes in important hormones and metabolites that favored this greater milk yield.

Therefore, the objectives of this research were to evaluate the influence of supplementing multiparous Holstein cows fed *ad libitum*, with low doses of recombinant bST (10.2 mg/day, 0.4 ml/14 d of POSILAC®, Monsanto Company, St. Louis, MO) during the peripartum and/or postpartum periods. Evaluation criteria for supplementation were milk production and composition, changes in BW and BCS, calving variables, plasma concentrations of specific hormones and metabolites, changes in liver metabolism of lipids and carbohydrates, and the incidences of calving-related diseases.

CHAPTER 2
LITERATURE REVIEW

Dry Matter (Feed) Intake during the Transition Period

Adequate nutrition during the transition period is of paramount importance for the dairy cow, as previously mentioned. Among the many aspects related to nutrition, dry matter intake (DMI) is fundamentally important because it establishes the amount of nutrients available to an animal to maintain health and to support milk production (National Research Council [NRC], 2001), and the magnitude and duration of the need of the animal to draw upon labile body reserves to meet her requirements for specific nutrients and energy. Despite the increased requirements for energy and nutrients, prepartum and postpartum DMI of dairy cows is not optimal. During the prepartum period, DMI decreases gradually as cows approach time to calve (Bell, 1995), and after calving DMI does not meet the requirements for the copious production of milk during the early weeks of lactation (Bauman and Currie, 1980; Drackley, 1999).

A number of studies have been carried out during the past several decades that evaluated the pattern of DMI during the transition period (Coppock et al., 1972; Marquardt et al., 1976; Bertics et al., 1992; Vazquez-Anon et al., 1994; Grum et al., 1996; Greenfield et al., 2000). General observations were that a gradual decline in DMI began about 3 wk prepartum, with the most dramatic decrease occurring during the final week preceding calving (Grummer, 1995). Marquardt et al. (1976) observed a decrease in DMI beginning at 7 d before calving in both young (first calf heifers, average age 28.4

mo) and aged (multiparous, average age 69.8 mo) cows. The DMI for heifers and cows on d 1 prepartum was 13% less than the DMI (% BW) observed at 7 d prepartum. These results indicated that DMI decreases in dairy cows occurred irrespective of their age (heifer and cows) around calving. Observations by Ingvartsen and Andersen (2000) also showed that significant decreases in DMI occurred in both heifers and dairy cows around parturition. The percentage decrease observed was ~ 20% in both animal classes when compared to -21 d DMI.

Greenfield et al. (2000) reported a greater depression in prepartum DMI of Holstein dairy cows. In their experiment, the decrease was about 30% and began at 7 to 10 d before calving. However, in a study conducted by Vazquez-Anon et al. (1994), the decrease in prepartum DMI was not seen until 2 d before calving; extent of depression was 40% when compared to the previous day DIM (d 3 prepartum). Recent results (Gulay et al., 2003a) reported that the decrease in DMI began 8 d prepartum, but the decrease only was significant during the last 4 d before calving. The mean DMI depression among the groups of cows was from 32 to 45% compared to levels observed at d 8 prepartum.

With regard to the postpartum DMI, many observations indicated a substantial increase following parturition (Grummer et al., 1995; Grum et al., 1996; Garcia-Gavidia, 1998; Santos et al., 1999; Greenfield et al., 2000; Doepel et al., 2002; Gulay et al., 2003a; McNamara et al., 2003, Selberg et al., 2004; Gulay et al., 2004), but the requirements for milk production in these studies also were far greater than the amount of nutrients ingested. Overall, during the early postpartum period, dairy cows show a sudden and marked increase in nutrient requirements at a time when DMI lags far behind (Bell, 1995;

Drackley, 1999). This leads to a period of negative energy balance (NEB), during which the dairy cow must rely on the mobilization of body tissue reserves (adipose tissue and protein from muscles) to meet her nutrient demands (Staples et al., 1990; de Vries and Veerkamp, 2000; Reist et al., 2002; Gulay et al., 2003a).

In a comprehensive study designed to investigate the general pattern of energy balance (EB) during early lactation in dairy cattle, de Vries et al. (1999) observed that maximum energy requirements for milk production occurred at wk 7, 5 and 6 for first, second and third lactation cows. On the other hand, averaged among all cows, maximum energy intake that would be sufficient to meet the demands occurred only by 72 DIM. In other studies, positive EB was achieved by d 22 (Santos et al., 2000), d 85 (Walters et al., 2002) and d 42 (Gulay et al., 2003a) of lactation. Despite divergent results with regard to time to achieve positive EB, all studies indicated that dairy cows experienced a period of NEB after calving.

The causes of the prepartum decrease in DMI are not well described or well understood. Due to the dramatic physiological changes occurring during the transition period, Forbes (1971) speculated that significant endocrine changes, such as the increase in estrogen around calving, were the likely causes for the depression in DMI. To evaluate this, he studied the effect of estrogen injections on voluntary intake of hay and concentrate diets by sheep. When animals were injected with quantities of estrogens that produced similar blood concentrations of the hormone to those found during late pregnancy, no significant effects were observed for hay intake; however, there were significant decreases in concentrate intake. This was the first evidence that estrogens could be responsible, at least in part, for the decrease in DMI around parturition.

To evaluate if elevated estrogen and other traits of late pregnancy were associated with depressed DIM during the transition period of Holstein cows, Erb et al. (1982) conducted an extensive statistical analysis using data from 86 multiparous cows. Their objective was to establish some associations between DMI (as percentage of BW) during the last week before calving with many variables, including the hormones estrone and estradiol α and β. Cow age and quantitative indices of seasonality (temperature, photoperiod, and plasma PRL) were negatively associated with percentage DMI, but none of the other hormones in plasma were associated with DMI (as percentage of BW).

More recently, Grummer et al. (1990) reported interesting results of a field study designed to evaluate the role of estrogen on ruminant DMI. Their experiment consisted of injecting nonpregnant lactating (260 d of lactation) and nonlactating Holstein cows with estradiol-17β benzoate during 14 d of feeding, followed by 3 d of fasting, to mimic the prepartum transition period. In the lactating group, estrogen injected cows showed decreased DMI, but not the controls. The decrease was 39% compared to baseline pre-injection period values of the lactating cows. Because milk production was negatively impacted by estrogen (decrease of 65% in production compared to baseline values), it was not possible to determine if the DMI depression was due to direct effects of estrogen; however, the non-lactating group of cows showed no effects of estrogen injections.

Goff and Horst (1997) proposed a different reason for the decrease in DMI observed shortly before calving. Their proposed reason for decrease was based on the concentrations of endogenous opioids (β-endorphin) seen around parturition. Plasma concentrations of these peptides typically increase prepartum to reduce the perception of pain by the cow during this phase. Because these compounds are used in treatment of diarrheal disease in humans to decrease the motility of the gastrointestinal tract, they may have a similar effect on the dairy cow, with one effect reflected as decreased DMI.

Other possibilities for causes of depression in DMI were evaluated by Hayirli et al. (2002). In their study, data from 699 Holsteins fed 49 diets during the final 3 wk of gestation were compiled from 16 experiments conducted during the 1990s. Objectives were to assess associations among animal factors (parity and BCS) and dietary factors (concentrations of organic macronutrients NE_L, CP, RUP, RDP, NDF, NFC, ADF, EE and ash concentrations) with the DMI depression observed during the prepartum phase of the transition period. The DMI (% of BW) decrease prepartum was positively correlated with parity and NFC, and negatively correlated with BCS, NDF and EE. Among all causes, day of gestation, other animal factors and dietary factors accounted with 56.1%, 19.7% and 24.2%, respectively, in the variation of DMI. The DMI decreased 32.2% during final 3 wk of gestation, and 88.9% of that decline occurred during the final week of gestation, which essentially agreed with previous findings. Average daily DMI during the final 3 wk of gestation for cows was greater than for heifers (1.88 vs. 1.69% of BW, respectively). These results indicated that pregnancy and animal factors (physiological factors) were the main reasons for decreased DMI observed approaching parturition; and that those from nutritional origin accounted for only part of the decrease and certainly played a less important role.

Clearly, results described and evaluated previously indicated that the transition period is marked by nutritional challenges. The hallmarks were the decrease of DMI when parturition was imminent (last week before calving), and the sluggish increase in DMI during the first weeks of lactation, which is a period of tremendous demands for nutrients. Despite extensive efforts to understand the physiological basis for these events during the last several decades, the causes for the depression in DMI during the prepartum transition period still are unknown. Some significant associations between variables of seasonality, animal factors (BCS) and a few dietary factors were identified, but the real underlining reason(s) for such behavior remains to be discovered.

Changes in Body Weight and Body Condition Score during the Transition Period

The decrease in DMI seen during the transition period triggers a cascade of metabolic responses in dairy cows. Among these is the mobilization of nutrient precursors from body reserves (adipose tissue [NEFA] and muscles [AA]) to meet the demands (Bell, 1995). If dairy cows are either mobilizing (decreasing) or increasing their body reserves, changes in body weight will occur. However, as Klosterman (1972) indicated, the assessment of body weight changes alone does not account for factors other than weight and frame size, and it is not the most suitable way to observe changes in cow metabolism. Therefore, in addition to BW measurements, it is important to evaluate body condition (BC), or status of cow fitness pertaining to degree of body fat (Wildman et al., 1982).

Useful systems developed to define means to score body condition, or define body condition score (BCS) of dairy cows in the US have been proposed by Wildman et al. (1982), Edmonson et al. (1989) and Ferguson et al. (1994). The first system proposed use of a scale from 1 to 5 to score thin (underconditioned) and fat (overconditioned) cows, respectively. Subsequent systems used the same scale with the inclusion of 0.25 point increments between scores, thus functioning as a 17-point scale. Despite these differences in the scoring systems, all essentially achieved the same objective of permitting BCS of dairy cows to be described during all phases of lactation, including the dry period.

The assessment of BW and BCS changes in dairy cows has been the focus of attention in a great number of experiments. Investigators were interested to observe whether these changes were positively or negatively associated with milk yield (Wildman et al., 1982; Stanisiewki et al., 1992; Domeq et al., 1997), reproductive performance (Studer, 1998; Formigoni and Trevisi, 2003) or health (Gearhart et al., 1990; Smith et al., 1997). With regard to transition cow studies, there also was a great interest in evaluating BW and BCS changes, because the BCS assesses the amount of metabolizable energy stored in fat and muscles (Edmonson et al., 1989), an important qualitative method to indirectly monitor nutritional status of transition cows.

Due to the nutritional challenges that dairy cows face during the transition period, changes in BW and BCS are expected during this period. General observations are that both BW and BCS would be lost beginning at calving and this loss continues through 4-8 wk of lactation, a period when dairy cows reach positive energy balance (Fronk et al., 1980; Doepel et al., 2002; Walters et al., 2002; Pushpakumara et al., 2003). Expulsion of the calf and fetal membranes at time of calving and the dramatic decrease in DMI observed around calving are two of the causes responsible for these losses (Smith et al., 1997). The extent of BCS loss reported by some authors varied from 0.2 to 1.05 pt, and it was correlated with BW loss. It has been estimated that a change in one unit of BCS corresponds to 56 kg of live weight change in the dairy cow (Otto et al., 1991). Because each 1-kg BW loss corresponds to -4.92 Mcal of NE_L (National Research Council [NRC], 1989), the energy loss from body reserves can be calculated by monitoring BCS scores.

Typically, cows lose from 55 to 289 Mcal of NE_L (BCS loss of 0.2 and 1.05 pt) during the first 4-8 wk of lactation, as mentioned previously.

It is after these first weeks postpartum that dairy cows begin to recover the BCS (energy) and BW lost during early lactation. Most authors (Staples et al., 1990; Rukkwamsuk et al., 1999; Pushpakumara et al., 2003) have shown that moderate changes occur after cows reach a state of positive energy balance (de Vries et al., 1999). Wildman et al. (1982) showed that relative to days in milk (DIM), dairy cows at less than 80 (n=570), between 80 and 159 (n=595), between 160-239 (649), and greater than 239 DIM (n=797) had average BCS of 2.51, 2.70, 2.95 and 3.39 pt, respectively. During the dry period, average BCS was 3.37 pt. for a total of 462 cows evaluated.

However, it is important to mention that it is during the dry period, of which about one-half is the prepartum phase of the transition period, that dairy cows recover from the extensive metabolic load imposed during the previous lactation. It also is during this time period that they must adjust their physiological systems to have a smooth transition from parturition to the next lactation (Bauman and Elliot, 1983). Therefore, recommendations are that dairy cows should maintain a BCS between 3.25 and 3.75 pt (average of 3.5 pt) during the ~60 d dry period and to recover about 0.25-050 pt that was lost during the early lactation period (Wildman et al., 1982; National Research Council [NRC], 2001). This recommended calving BCS would indicate that they have replenished their body reserves, and also would avoid excess deposition of adipose tissue, which often has been linked with the increased incidence of health disturbances postpartum (Gerloff, 1986; Maisey et al., 1993; Laven and Andrews, 1998).

If dairy cows are underconditioned during the dry period, losses in BCS actually can begin to occur before calving, as demonstrated by Domeq et al. (1997). In their study, early dry period dairy cows, with an average BCS of 2.77 pt (0.58 pt less than the recommended [Wildman et al., 1982]) began to lose BCS during the last 2 wk before calving. The BCS at parturition averaged 2.66 pt, and mean loss of BCS during the first 4 wk of lactation was 0.62 pt, or ≅ 170.82 Mcal of NE_L. This showed that dry-period undercondition was not desirable for transition dairy cows and led to loss of BW during the prepartum transition period. This also led to dairy cows being more vulnerable to metabolic disorders, such as fatty liver (Morrow, 1976; Grummer, 1993).

The extent of BCS loss after calving varied among cows with different BCS at parturition. Gerloff et al. (1986) observed that dairy cows with greater BCS at calving (3.29, 3.03 and 2.97) had greater BCS loss after parturition (1.17, 0.8 and 0.72, pt, respectively). Pedron et al. (1993) observed a similar pattern of BCS loss after parturition. In their study, dairy cows with BCS of 4.0, 3.5 and 3.0 at calving had greater loss of BCS during the first 8 wk of lactation (1.05, 0.8 and 0.6 pt, respectively). However, Smith et al. (1997) did not find any correlation between BCS at parturition and BCS loss during the early postpartum of obese cows (BCS 4.3) and cows with the recommended BCS (3.5). Even under their treatment regimens, which included restricted feeding of obese and normal BCS groups from 14-45 DIM, overall BCS losses were about 0.90 pt during the first 45 d of lactation.

Losses of BCS during the early postpartum period did not seem to impact milk yield. Pedron et al. (1993) obtained results that suggested that overconditioned dairy cows at calving (4.5 BCS) did produce more milk from 15-60 DIM than cows with BCS of 3.0 and 3.5. The milk yields were 37.54, 39.86 and 45.07 kg/d for cows with BCS of 3.0, 3.5 and 4.5, respectively. Despite the numerical differences, the milk yield differences across groups were not significant. Others reported that overconditioned cows or those with recommended BCS at calving produced the same amount of milk during early lactation, and also that loss of BCS did not impact milk production. In a study conducted by Fronk et al. (1980), control and overconditioned cows at calving (BCS 3.0 and 3.8 pt., respectively) had BCS losses of 0.36 and 0.26 pt. during 14-63 DIM. The MY for both groups was ≅ 34.5 kg/d during this period. In addition, Smith et al. (1997) reported that average MY did not differ among obese and normal-conditioned cows during the first 42 DIM. The BCS loss and average MY across groups during the period was ~0.9 pt and 38 kg/d. Similarly, Busato et al. (2002) also observed that significant BCS loss during the first 56 DIM (≥ 0.75 pt.) did not impact MY of fat and lean cows (BCS > or < than 3.25 prepartum). Average MY of treatment groups was 28.1 kg/d throughout experimental period.

At the physiological level, BCS losses have been correlated with concentrations of NEFA in plasma (Bauman and Currie, 1980). During the transition period, there was increased mobilization of adipose tissue from body stores beginning one week before parturition, regardless of the BCS status (underconditioned, normal or overconditioned; Santos et al., 2000; Busato et al., 2002; Gulay et al, 2003a). Another important observation was that postpartum concentrations of NEFA in plasma of overconditioned cows were significantly greater during the first weeks following calving than in cows with adequate BCS. Possible reasons for differences were because these cows had greater stores of body fat available to be mobilized, and/or due to differences in the metabolic adaptations that had occurred during the transition period, particularly beginning at the days (2-3) before parturition.

With regard to BCS and health, risks of disease incidences are strongly associated with BCS during the prepartum period and with losses of BCS during the early postpartum period. Morrow (1976) was one of the first investigators to observe that overfeeding dairy cows during the dry period elevated the risk factor for the incidence of "fat cow syndrome". This syndrome is related to the excessive mobilization of adipose tissue during the transition period, and always has disastrous consequences for the dairy cow (Grummer et al., 1993). Cows with this syndrome usually had one or more of the following disorders: milk fever, ketosis, displaced abomasum, indigestion, retained fetal membranes, metritis, mastitis or salmonelosis (Morrow, 1976).

Subsequent studies (Gearhart et al., 1990; Heuer et al., 1999) reported that when cows were overconditioned at dry-off they had more reproductive and foot problems. Overconditioning during early lactation was related to higher incidence of metritis and milk fever, and that BCS loss during the dry period also was associated with dystocia. For subsequent reproductive efficiency, it has been proposed that serious energy deficit during the postpartum transition period and early lactation negatively impacted the conception rate at first insemination, number of days open, and number of inseminations to conception for dairy cows. This energy deficit led to decreased blood concentrations of insulin, insulin-like growth factor-I, and progesterone, which had carryover effects on postpartum ovarian activity (Staples et al., 1990; Formigoni and Trevisi, 2003; Pushpakumara et al., 2003). Because the BCS/weight loss seen during the early postpartum period occurs due to an energy deficit, it has been directly correlated with reproductive performance of dairy cattle during the early postpartum period.

The assessment of BCS during the transition period is an important method to critically evaluate the nutritional status of dairy cows. The metabolic changes of the transition period imposes changes in BCS, and by the monitoring these, it has been possible to manage cows accordingly to improve productivity, reproductive performance and minimize the incidences of health disturbances.

Hormonal and Metabolic Adaptations during the Transition Period

The loss in BCS during the transition period is a reflection of the metabolic adaptations of many systems, organs and tissues of the dairy cow to cope with the increased requirements for nutrients. At the onset of calving, the mammary gland becomes the physiological priority, and because the nutrient input via feed intake is not optimal to meet the demands for milk production (pattern of DMI and nutrient requirements), a series of metabolic adaptations occurs to allow the cow to meet the needs. Loss in BCS indicates mobilization of energy sources from adipose tissue, the main energy storage tissue in the body, and lactate/ amino acids from other body tissues, all to be used primarily as gluconeogenic precursors by the liver. However, the mobilization of these precursors does not occur as a random event, rather, it is highly coordinated and is orchestrated by hormonal changes that occur during this phase (Bauman and Currie, 1980; Chilliard, 1999).

The key event in the metabolic adaptation seen during the transition period is the change in function in the adipose from anabolism (synthesis of triacylglycerol from glucose, and storage of triacylglycerol), to catabolism [breakdown of triacylglycerol, and release of glycerol and fatty acids (NEFA) into the blood stream; Bauman and Elliot, 1983]. These metabolic changes, which begin before calving (Grummer, 1995), also were observed in adipose tissue collected from dairy cows 2 wk after calving (early lactation). The general observations observed *in vitro* were that adipocytes (functional unit of the adipose tissue) obtained from lactating cows were smaller in size, had increased glycerol release when stimulated by adrenaline, and also had decreased uptake of glucose from the media when compared to the cells obtained 2 wk before parturition. In addition, blood samples obtained from cows during the early lactation period had increased concentrations of NEFA, confirming the suspicion of increased mobilization of lipids from adipose cells (Pike and Roberts, 1980).

In a series of experiments designed to evaluate adipose tissue metabolism of dairy cows overfed or restricted fed during the dry period, Rukkwamsuk et al. (1998, 1999) observed that basal *in vitro* lipolytic rate, as measured by glycerol production per gram of adipose tissue of restricted fed cows, was greater at 1 wk before parturition compared to 0.5, 1, 2 and 3 wk after parturition. This increase was accompanied by greater mean plasma concentrations of NEFA during wk 0.5 and 1 than during wk 2, 3 and 6. In addition, the *in vitro* rate of esterification (measured by the formation of labeled TAG using labeled oleate) was significantly less at wk 1, 2 and 3 after parturition than at 1 wk before parturition. The decrease in basal rate of esterification at 0.5 wk was 69% and 81% for cows that were restricted fed and overfed, respectively. These results corroborated previous observations, which showed increased lipolytic activity and a decreased lipogenic activity in adipocytes of dairy cows during the transition period.

In addition to the direct changes observed in the adipose tissue activity, others also observed changes in concentrations of NEFA in plasma around parturition (Simmons et al., 1994; Smith et al., 1997; Santos et al., 2000). In these studies, concentrations of NEFA in plasma began to increase at 1 wk before parturition, peaked 1 wk after parturition, and decreased sharply as lactation progressed. Mean concentrations of NEFA in plasma were about 200, 1,000-1,200, and 400 μeq/L during prepartum, at 1 wk postpartum and during 14-50 d after parturition, respectively.

To better understand what might be controlling the described changes, it is important to highlight the basic metabolic pathways that occur in the adipocyte with regard to lipid metabolism. The adipocyte synthesizes lipid (TAG) using fatty acids that are synthesized either *de novo* within the cells (mostly from glucose or acetate) or from fatty acids released from blood TAG of chylomicrons and very low density lipoproteins (VLDL) by the action of lipoprotein lipase (LPL). Fatty acids are released from stored TAG within the adipocyte (lipolysis) by the action of the hormone sensitive lipase enzyme. Some of the fatty acids released are re-esterified within the adipocytes, but the remainder are released and transported into the blood for use elsewhere in the body (Figure 1).

The controllers of these pathways are hormones and other humoral factors. The adipose tissue of dairy cows is mainly lipogenic when stimulated by insulin and in the presence of glucose (Bauman and Elliot, 1983; Kersten, 2001), and lipolytic when stimulated by glucagon, catecholamines such as epinephrine (β-adrenergic receptor stimulator), and adenosine (McNamara, 1991; McNamara et al., 1992; Vernon and Pond, 1997; Chilliard, 2000).

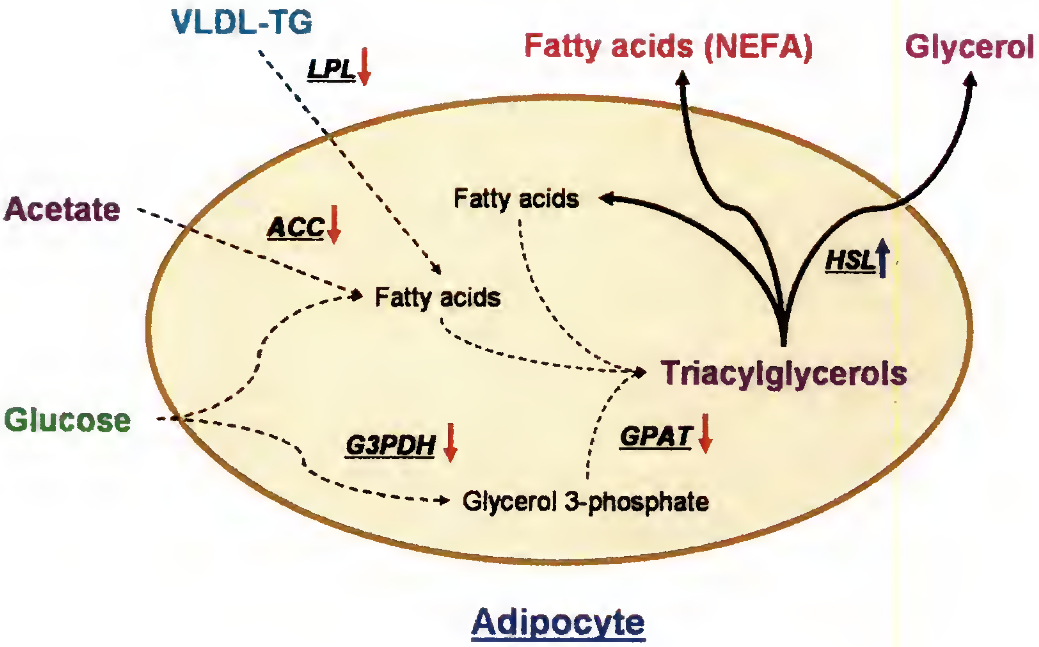

Figure 1. Some metabolic pathways within adipocytes and their adaptations during the transition period. Enzymes: ACC, acetyl CoA carboxylase; LPL lipoprotein lipase; G3PDH, glycerol 3-phosphate dehydrogenase; GPAT, glycerol 3-phosphate acyltransferase; HSL, hormone-sensitive lipase. Enzyme activity: ↑ or ↓ (increased or decreased, respectively). Metabolic fluxes: bold line, flux increased; dashed line, flux decreased (Adapted from Vernon and Pond, 1997).

Attempts to identify the role of these hormones in controlling lipid metabolism in the adipocyte and in overall body began during early 1970's. Smith et al. (1975) conducted an experiment to monitor changes in blood concentrations of insulin and somatotropin beginning at parturition through 56 DIM, to relate these changes to the nutritional status of dairy cows during the early postpartum period. Observations were that mean concentrations of insulin and glucose were at their lowest from 1-20 DIM (8.8 μU/mL and 37.3 mg/dL, respectively) and had a constant increase thereafter. From 21 to 40 DIM mean concentrations were 10.3 μU/mL and 40.6 mg/dL, and from 41 to 56 DIM, mean plasma concentrations of insulin and glucose were 11.9 μU/mL and 43.3 mg/dL, respectively. However, mean concentrations of somatotropin were greatest at calving

(19.3 ng/mL), and decreased as lactation progressed through 56 DIM (13.6 ng/mL). In another study, Lomax et al. (1979) showed that arterial and portal-venous concentrations of insulin, and the pancreatic output and hepatic uptake of insulin were approximately 2, 3, 3 and 5-fold greater in normally fed non-lactating cows than in lactating cows. This confirmed that differences in the secretion of insulin existed during the two physiological states.

Similar results also were observed in subsequent studies conducted to evaluate metabolic profiles of dairy cows during the transition period (Athanasiou and Phillips, 1978; Putnam and Varga, 1999; Busato et al., 2002, Block et al., 2003). In those studies, mean concentrations of insulin during the prepartum and postpartum phases of the transition period were about 0.7-0.8 and 0.4 ng/mL; and for somatotropin, mean prepartum and postpartum concentrations were 8.0 and 14.0 ng/mL, respectively. With regard to concentrations of somatotropin after parturition, they continued to increase, or were greater as lactation progressed compared to those concentrations seen prepartum.

The decrease in concentration of insulin during the prepartum period seems to be responsible for increased lipolysis in the adipose tissue. However, Rukkwamsuk et al. (1999) also observed that adipocytes collected from dairy cows at 0.5, 1 and 3 wk postpartum had significantly lower *in vitro* rates of esterification than prepartum cells even when both types of cells (prepartum and postpartum) were treated with insulin and glucose in the media. In addition, rates of lipolysis increased significantly when adrenaline was added to the media (Pike and Roberts, 1980). This indicated that there also was altered responsiveness of adipose tissue to insulin and other effectors around parturition.

In an elegant experiment, Jaster and Wegner (1981) observed that the lipolytic responsiveness seen during early lactation was greater than during the dry period due to the increased number of β-adrenergic receptors (lipolytic) located in the cell surface. Subsequently, Bauman and Elliot (1983) also reported that the *in vivo* lipolytic response of adipose tissue to adrenaline during early pregnancy was greater than during late pregnancy in dairy cows. The changes in lipolytic responsiveness were attributed to the hormonal changes observed around parturition, which included decreased P_4 and increased estradiol, prolactin, and PGF2α concentrations during the prepartum period (Thatcher et al., 1980; Bauman and Elliot, 1983). In addition to these hormones, the increase in somatotropin also can be considered as a regulator of lipolysis because the hormone increases adipose tissue response to catecholamines, and it is a potent inhibitor of lipogenesis (Bauman and Vernon, 1993). Therefore, changes in catecholamine response indicates that the system is sensitive to the hormonal changes described, and when it is driven towards lipolysis, it becomes resistant (less responsive) to insulin stimulation of lipogenesis (Vernon and Pond, 1997).

Another key event in the metabolic changes seen during the transition period is the adaptation seen in carbohydrate and protein metabolism. Bell (1995) estimated that for dairy cow producing 30 kg of milk/d at 4 d postpartum there is a deficit of 500 g/d of gluconeogenic precursors (propionate, dietary amino acids, lactate, and glycerol from adipose tissue) that are needed to meet daily demands for glucose. When the supply of propionate for hepatic gluconeogenesis is limiting, as seen during the time there is a decrease in DMI, lactate and AA released from muscles are used as gluconeogenic substrates (Baird et al., 1980).

To observe the mobilization of AA from body tissues around parturition, Blum et al. (1985) assessed blood concentrations of 3-methylhistidine (3-MH) during the transition period. The 3-MH is a by-product formed by methylation of histidine in actin and in myosin of muscles during the accretion of protein that occurs physiologically. When there is a breakdown of these macromolecules, 3-MH is liberated into the AA pool in the blood. Results reported were that plasma 3-MH increased rapidly before calving (20 μmol/L) to maximal concentrations at 1 wk postpartum (32 μmol/L), thereafter decreasing to a low of 14 μmol/L at 5 wk after calving. All indicated that there was increased mobilization of proteins from body reserves during this period. Bell and Bauman (1997) observed a similar pattern in changes of 3-MH concentrations of mature Holstein cows around parturition. Despite differences in magnitude of absolute values observed during the previous study, 3-MH concentrations were about 4, 13 and 4 μmol/L at d 17 prepartum, and at d 4 and 17 postpartum, respectively. Although these results cannot determine if AA mobilized are from skeletal muscles or from other tissues, such as the involuting uterus, information reviewed by Bell (1995) suggested that the skeletal muscle tissue strongly contributed to the blood plasma AA pool. He also indicated that the research reported by Reid et al. (1980) showed a significant reduction (25%) in muscle fiber diameter of dairy cows immediately after calving. Therefore, there was strong evidence to indicate that there would be an increase in lactate and AA availability in the blood from muscles for potential use by the liver for glucose production.

To evaluate if there was increased use of lactate and AA as gluconeogenic precursors by the liver, Greenfield et al. (2000) assessed the abundance of pyruvate carboxylase (PC) mRNA and its activity in livers of dairy cows during the transition period. They evaluated the PC enzyme because it is a rate limiting enzyme for hepatic gluconeogenesis from the precursors lactate, pyruvate, alanine and other AA. Liver biopsies were conducted at d -28, -14, +1, +28 and +56 relative to parturition. Abundance of mRNA for PC increased significantly (7.5-fold) the day after calving compared to d -28 levels. Also, the activity of the enzyme increased linearly as mRNA abundance increased. It is not possible to quantify how much of the AA mobilized contributed to hepatic gluconeogenesis (Bell and Bauman, 1997), but there is strong evidence indicating that the liver adapts around calving to utilize AA and other sources to synthesize glucose via gluconeogenesis.

Among the hormones that play a role in controlling these metabolic adaptations around parturition, insulin, corticoids and somatotropin can be considered to have important functions. The decrease in insulin concentration during early lactation diminishes the capacity for glucose uptake by tissues that are insulin-dependent, such as skeletal muscles, and this can initiate a cascade of events leading to protein breakdown and AA release into the blood stream (Bauman and Vernon, 1993). Corticoids are potent stimulators of hepatic gluconeogenesis in lactating and non-lactating cows. Because the concentration of corticoids increases around calving, it may be associated with the increase in gluconeogenic activity. In fact, these assumptions were made based upon results of studies conducted during the late 1940’s and early 1950’s that addressed the treatment of ketotic cows with cortisone (steroid hormone; Dye et al., 1953). Observations were that after the administration of the steroid to ketotic cows, there was mobilization of amino acids from muscles, increased liver deamination of these amino acids, increased urea production, and increased gluconeogenesis from some of the resultant ketoacids.

With regard to somatotropin, results from numerous studies have evaluated the effects of exogenous recombinant bovine somatotropin (rbST) in lactating dairy cows and other ruminants. Results indicated that somatotropin exerted direct and/or indirect actions (through insulin-like growth factors) to regulate the changes described previously (Bell and Bauman, 1997). Because there is an increase in the concentration of somatotropin as lactation progresses, it is a strong candidate as mediator of these changes.

The onset of lactation also requires metabolic adaptations of tissues other than adipose, muscle and liver to help meet the demands for another important nutrient, calcium (Ca; Horst, 1986; Horst et al., 1994; Goff and Horst, 2003). The initiation of copious milk production includes secretion of great quantities of calcium in the milk (National Research Council [NRC], 2001). In addition to the sharp increase in demand for calcium as lactation is initiated and progresses, the slow increase in DMI has a negative impact on mineral metabolism during this time period (Gerloff, 1988). When this occurs the dairy cow must rely on the mobilization of calcium from bones to meet its needs (Bauman and Elliot, 1983). However, following the onset of lactation, there is a delay of more than a week before bone calcium is mobilized extensively (Ramberg et al., 1970). The great output of calcium in the milk leads to decreased blood concentration of the mineral, a condition that is clinically manifested as parturient paresis, or milk fever (Kronfeld, 1971).

Recent research involving calcium metabolism of transition dairy cows has explored the potential of using prepartum transition diets varying in the cation-anion

(DCAD) difference to improve the adaptation described as lactation occurs (Moore et al., 2000; Melendez et al., 2002; Gulay et al., 2003a; Melendez et al., 2003). The dietary DCAD relates to the difference between total fixed (nonmetabolizable and bioavailable) cations and total fixed anions in the diet, which most often is calculated as meq (Na + K) – (Cl + S)/100 g of dietary DM. Negative and positive DCAD diets are denominated anionic and cationic, respectively. Feeding prepartum diets with negative DCAD causes mild metabolic acidosis, which increases calcium excretion via urine (Gaynor et al., 1989). This, in turn, stimulates the hormonal system that controls calcium metabolism (parathormone (PTH), and vitamin D) to increase absorption of calcium from the intestines and bone resorption of calcium (Horst, 1986; Wang et al., 1994).

Anionic diets are efficient in maintaining blood calcium levels around parturition in mature cows and in heifers, as demonstrated by Moore et al. (2000). In their study, heifers and cows fed anionic (-15 meq/100 g of dietary DM) or neutral (0) DCAD diets had higher concentrations of ionized calcium (iCa, the biologically active form of the mineral; Dauth et al., 1984) in blood than those fed a cationic diet (+15 meq/100 g DM) during prepartum period and at calving. The iCa concentrations ranged from 3.67 to 4.95 mg/dL, or about 50% of the total concentration of calcium in blood, which ranges from 9 to 11 mg/dL (Kronfeld, 1971). Despite differences in calcium concentration, no differences in plasma hydroxyproline (blood metabolite released from bones when there is tissue resorption) were observed among experimental groups, which indicated that their anionic diet did not cause extensive bone resorption. However, Gaynor et al. (1989) demonstrated that anionic diets caused calcium mobilization from bone, as well as an increase in excretion of calcium in the urine.

These are just a few examples of the many studies carried out to assess the metabolic adaptations that take place during the transition period. In general, all agree that major changes in lipid, carbohydrate, protein and mineral metabolism occur in the dairy cow to accommodate the demands brought about by the abrupt onset and rapid increase in milk production. The changes in hormonal concentrations seen around parturition likely orchestrated the adaptations, which, it seemed occurred to the benefit of the new born at the expense of the dairy cow.

Liver Function during the Transition Period

The primary fate of NEFA mobilized from adipose tissue and lactate/AA from muscles is uptake and metabolism in the liver. The liver plays a unique role in processing these precursors to produce glucose to support mammary metabolism and milk synthesis and also other molecules that can be used as energy sources by the peripheral tissues, such as ketone bodies (Grummer, 1993). In the dairy cow, it was estimated that 85% of all glucose produced in the body came from the liver, with the remainder being synthesized in the kidney (Bauman and Elliot, 1983). This underlined the importance of this organ in the metabolism of carbohydrates, especially during the transition period. Besides being the center of metabolism of carbohydrates, lipids and proteins, the liver is the center of defense, it is the control station of the hormonal system (it executes the clearance of hormones secreted by other endocrine glands), and it has an important role in maintaining blood pH and immunoregulatory functions (Kmieć et al., 2001).

At the microscopic level, the liver is composed of hepatocytes, non-hepatocyte cells (sinusoidal endothelial, Kupffer, stellate and pit cells), and an extracellular space compartment (sinusoidal lumen, disse space and biliary canaliculi). In the rat, these structures occupy 77.8, 6.3 and 15.9%, respectively, of the total volume of the organ. The hepatocytes execute the major metabolic functions described previously, Kupffer cells are hepatic macrophages, stellate cells are fat-storage cells or vitamin A rich cells, and pit cells represent liver-specific natural killer cells (NK, cancer killer; Figure 2).

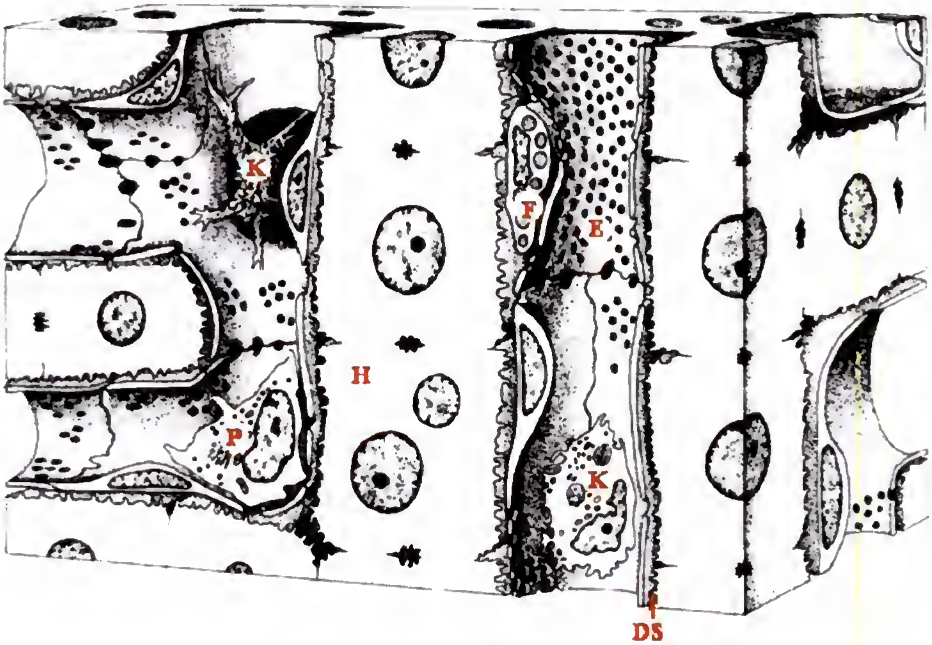

Figure. 2. Schematic drawing of the rat liver tissue. The parenchyma is built up of a trabecular network of cell plates made up of hepatocytes (H). The wall of sinusoids, formed by fenestrated endothelial cells (E) and Kupffer cells (K), does not show basal membrane; however, stellate cells (F) are present in the perisinusoidal space of Disse (DS). Pit cells (P) also stay attached to the sinusoid wall (Sasse et al., 1992; with permission).

In the dairy cow, the stage of production and the feeding behavior exert extensive changes in blood flow and types of metabolites that reach the liver, as observed by Lomax and Baird (1983). In their experiment, blood flow to the liver of normally fed lactating cows at 44 DIM was 52% greater than in non-lactating cows (average 11 mo after last parturition). Another observation was that after short-term fasting (4 d), a significant reduction (50%) in liver blood flow occurred, regardless of the milk production stage. During a normal fed state 46, 16, 8.6 and 0.8% of assimilated precursors propionate, lactate, AA and glycerol were converted to glucose, respectively, in the liver. After a 4-d period of fasting, contributions of these precursors to glucose production were 2.2, 81.6, 36.8 and 21.9%, respectively. In addition, after the 4-d fast, NEFA was 98.9% of the assimilated precursor converted to ketones, compared with 0.0% of the butyrate. It is likely, that to some extent, these changes also occur in transition dairy cows around parturition, a period characterized by a significant decrease in DMI and energy and precursor availability.

Liver gluconeogenesis is not a simple process. The pathway has irreversible glycolytic reactions, which requires specific, rate-limiting enzymes to catalyze them. The reactions are (1) the mitochondrial conversion of pyruvate to oxalacetate; (2) cytosolic conversion of oxalacetate to phosphoenolpyruvate; (3) cytosolic hydrolysis of fructose-1,6-biphosphate to form fructose-6-phosphate; and (4) cytosolic hydrolysis of glucose-6-phosphate to produce glucose. The rate-limiting enzymes are (1) *pyruvate carboxylase* (PC, EC 6.4.1.1; Greenfield et al., 2000); (2) *phosphoenolpyruvate carboxykinase* (PEPCK, EC 4.1.1.32, Lomax et al., 1986); (3) *fructose-1,6-diphosphatase* (FBPase, EC 3.1.3.11; Rukkwamsuk et al., 1999); and (4) *glucose-6-phosphatase* (EC 3.1.3.9; Rukkwamsuk et al., 1999). The precursors utilized in these pathways are glycerol, lactate, AA and the product of complete oxidation of NEFAs, acetyl CoA (Beitz, 1993; Zubay, 1998).

Once transported into the hepatocyte, NEFAs have three possible paths of metabolism. The first is complete oxidation in the tricarboxylic acid cycle (TCA cycle) reactions by the process of β-oxidation, generating CO_2, H_2O and ATP. The second is partial oxididation to acetyl-CoA, and then diversion via TCA cycle to form ketone bodies (acetoacetate, β-hydroxybutyrate and acetone). The third is re-esterification to form TAG molecules and storage in the cell or excreted as very low-density lipoprotein cholesterol (VLDL; Drackley, 1999). The steps of NEFA metabolism (in the hepatocytes) are regulated by a group of enzymes including *carnitine palmitoyltransferase I* (CPT-1, EC 2.3.1.21), which transfers NEFA into mitochondria for complete β-oxidation and ketogenesis. The other enzymes in this group are *3-hydroxy-3-methylglutaryl CoA synthase* (HMG-CoA, EC 4.1.3.5), the regulatory enzyme in mitochondrial ketogenesis, and enzymes of the esterification pathway, *glycerol-3-phosphate acyltransferase* (GPAT, EC 2.3.1.15), *phosphatidate phosphohydrolase* (PAPase, EC 3.1.3.4) and *diacylglycerol acyltransferase* (DGAT, EC 2.3.1.20). The third destination is highly regulated by *microsomal triacylglycerol transfer protein* (MTP, EC 5.3.4.1; White et al., 1998), which has a critical role in coordinating the assembly of VLDL in the liver, so that it can be secreted from the hepatocyte.

Lipid metabolism is a key area in the biology of the transition period of the dairy cow. Normal lipid mobilization from adipose tissue seems to cause no problems for liver function; however, excessive lipid mobilization is linked to greater incidences of periparturient health problems (Drackley, 1999). Histologically, detectable fat is present in the liver of virtually all dairy cows during the first weeks after calving, but the condition is considered severe if the percent of fat in liver cell is greater than 20% on a DM basis (Reid and Collins, 1980). In a survey study, Reid (1980) observed that 37, 48 and 15 % of Friesian cows, and 62, 33, and 5 % of Guernsey cows had mild (0-20), moderate (20-40) or severe (40-70) percentage of fat in liver on a DM basis, respectively. In addition, Pearson and Maas (2002) indicated that moderate to high amounts of fat (15-30%, wet weight basis) were present in the liver of all postparturient, high-producting dairy cows, even those that were healthy.

To determine whether the accumulation of fat in the liver is accompanied by significant changes in cell and organelle structure, Reid and Collins (1980) conducted an experiment to correlate percentage of fat in liver, indicators of cell function, and observations of histological and stereological analysis of the liver of dairy cows with mild (<20% fat) or severe fatty liver (>30% fat on a DM basis). Their observations showed that cows with severe fatty liver had a significant increase in plasma concentrations of the enzyme *aspartate aminotransferase* (AST, EC 2.6.1.1) compared to cows with mild fatty liver (92.7 vs. 49.9 IU/L), indicating liver damage. In addition, cows with severe fatty liver had deposition of fat cysts (lipogranulomas, that originated from ruptured stellate cells) in the hepatocyes, increased cell volume, decreased volume of RER per liver cell, and evidence of mitochondrial damage. All of these changes indicated significant morphological and structural changes in hepatocyte organelles due to excessive deposition of fat.

Because there is no limiting step in the uptake of circulating NEFA by the liver, excessive mobilization of fat from adipose tissue can lead to subsequent hepatic deposition of the metabolite in the form of TAG. Gaal et al. (1983) found a significant positive correlation between serum concentration of NEFAs and the total lipid concentration in liver (+0.67; $P<0.01$) of Friesian dairy cows during the transition period. However, no significant correlation was found between β-HBA and total liver lipid content, but there was a significant correlation between β-HBA and NEFA (+.032, $P<0.05$). Concentrations of total lipids in liver (wet basis), and NEFA and β-HBA concentrations in blood ranged from 3.5-20.1%, 249-1,888 µmol/L and 0.19-1.03 mmol/L, respectively. Ono et al. (1988) observed that peak concentrations of NEFAs of healthy Holstein cows at day of calving were significantly less than in diseased cows. The concentration of NEFA in plasma and the percentage of fat in liver (DM basis) of normal and diseased cows were 800 µEq/L and 1,400 µEq/L, 18.9 and 25.3%, respectively. Van Den Top et al. (1995) and Grummer (1993) agreed that rate of fatty acid esterification into TAG in liver was dependent upon the supply of fatty acids, as mentioned previously; however, Grummer (1993) also suggested that fatty liver occured when the rate of hepatic fatty acid esterification exceeded the rate of TAG disappearance via hydrolysis plus export as a constituent of VLDL.

Information cited previously indicates that the slow rate of TAG export as a constituent of VLDL may be one cause of fatty liver. In fact, Bauchart (1993) observed that hepatic VLDL secretion in calves was five-times less than in young adults (± 24 yr. old). Pullen et al. (1990) earlier conducted a study to compare the ability of liver slices from eight species to synthesize TAG from the NEFA in the incubation media. The rate of liver TAG synthesis was similar among species studied; however, liver slices from species in which the liver contribution to lipogenesis is minor (sheep, cattle, pig and guinea pig) secreted less TAG than liver slices from species in which lipogenesis occurs predominantly in the liver (chicken and fish) or in liver and adipose tissue (rat and rabbit). These results suggested that the ability of liver to secrete VLDL was proportional to the capacity of the liver to synthesize lipids, and that ruminants have an inherently low capacity to export TAG as a constituent of VLDL.

To test the hypothesis that rates of hepatic TAG esterification were positively correlated to concentration of NEFAs in plasma, Van Den Top et al. (1996) conducted an experiment to obtain information about the activities of the enzymes of the esterification pathway (GPAT, PAPase and DGAT) in Holstein x Dutch Friesian that developed fatty liver during postpartum. A surprising observation was that microsomal GPAT, the enzyme that catalyzes an early step in the first part of the pathway leading to TAG and phospholipid formation, was depressed in overfed cows, despite the higher concentrations of NEFA in plasma of these cows compared to restricted fed cows (1.50 vs. 0.75 mmol/L at 1.5 wk postpartum). Although GPAT activity was lower, TAG liver accumulation of overfed cows was greater than in restricted fed cows during the first 8 wk postpartum. The authors suggested that this depression in GPAT was a mechanism used to divert fatty acids from esterification to the β-oxidation pathway, so that further fatty liver development, and perhaps any associated liver damage, were diminished.

Neutral lipids and phospholipids comprise the total lipid deposited in the liver. Neutral lipids are mainly cholesterol esters, TAG, free fatty acids (FFA) and cholesterol. Phospholipids are composed by phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl choline and sphingomyelin. Ono et al. (1988) observed that the TAG portion of liver of Holstein cows that had 18.9 and 23.5% fat in liver (DM basis) was 4.5 and 9.1%, respectively. An interesting finding was that, in addition to these increase in the neutral lipids in the TAG component of diseased livers, there also was an increase in phosphatidyl serine in phospholipids. These results agreed with observations of Hippen et al. (1999). In their study clinically diseased cows with fatty liver had higher liver TAG than non-diseased cows, as expected. The percentage of TAG in diseased cows was greater than 8% of the total liver volume on a wet weight basis.

Some recent research involving fatty liver syndrome explored the use of glucagon to improve the condition in diseased Holstein cows (Bobe et al., 2003). The authors believed that glucagon would improve health status of diseased cows by decreasing lipolysis due to indirect actions via increased concentrations of plasma glucose and insulin in the adipocyte. Glucagon injections (5 mg every 8 hr [15 mg/d]) began at d 8 postpartum and continued through 22 DIM. Their results showed that injections of glucagon decreased plasma concentrations of NEFA, increased concentrations of glucose and insulin, and decreased liver TAG of older cows (>3.5 yr), but not of younger cows. No effects of glucagon injections on MY were observed. These results indicated that glucagon significantly decreased TAG accumulation in liver, by decreasing the mobilization of NEFA from adipose tissue, which agreed with the hypotheses they previously described.

Clearly, the liver has a major role throughout the transition period of dairy cows. The decrease in DMI and changes in metabolism around parturition have both direct and indirect effects on liver function. Decrease in blood flow and changes in the assimilation of precursors require that an adaptation in the hepatic gluconeogenic and lipolytic-ketogenic pathways occurs in order to use the available precursors. However, if fat mobilization exceeds that used by the liver, bad consequences likely will occur. Excessive fat deposition can cause structural damage in the liver, can lead to excessive production of ketone bodies, and can impair overall liver function. Studies undertaken to evaluate the basic mechanism of fat deposition in the cow liver indicated that the inability to limit fat uptake and the inherent decrease in capacity to excrete TAG via VLDL in ruminants were the likely causes for the deposition of fat in the liver. These changes ultimately will exert negative effects on overall health of the cow during the transition period.

Mammary Gland Function during the Transition Period

The metabolic adaptations during the transition period occur almost entirely to support the function of the mammary gland following parturition. However, it has been recognized that physiological changes that take place only during the dry period also are required for the appropriate lactogenic function of the mammary gland (Capuco and Akers, 1999). These changes occur during distinct phases or functional transitions in response to the cessation of milking, and to the dramatic variations in the concentrations of reproductive and metabolic hormones observed during the prepartum transition period (Tucker, 2000). For the dairy cow, the functional transitions are from lactation to forced involution (dry-off), from involution to colostrogenesis, and from colostrogenesis to initiation of the new lactation (Oliver and Sordillo, 1989). Early studies indicated that a dry period length of 40-60 d was necessary for these transitions to occur. However, results of more recent research suggests that a 30-35 d dry-period likely is sufficient for the optimal function of the mammary gland during the lactation that follows (Bachman, 2002; Gulay et al., 2003a).

The beginning of the dry period is characterized by a dramatic shift in the function of the mammary epithelial cells. This is reflected by changes in concentrations of constituents in mammary secretions, as were observed by Athie et al. (1996). In their experiment, Holstein cows assigned to a 60 d dry period showed a loss of differentiated function and overall functionality of the mammary epithelial cells (decrease in the volume and in the concentration of α-lactalbumin, lactose and citrate in mammary secretions), as well as an increase in the capacity of immune defense in the organ (increase in immunoglobulins, lactoferrin and somatic cell count in mammary secretions), as the dry period progressed. Changes were more evident after 7-10 d dry and were maintained through the end of the experimental period (30 d dry). This shift in function likely was initiated by the increase in alveolar pressure and by accumulation of the feedback inhibitor of lactation caused by milk stasis (Fleet et al., 1978; Wilde et al., 1995).

In addition to the changes in the function of mammary epithelial cells, there also are significant effects of dry-off on the structure of the mammary parenchymal tissue, as observed by histological studies. In non-pregnant crossbred beef cows, non-suckling of an udder-half for 21 or 42 d (mimicking a dry-off) caused a 50 and 64 % reductions of total parenchymal DNA compared to the continuously suckled udder-half. Despite the histological evidence of alveolar structure in non-suckled glands, numbers of cells per alveolar cross-section were reduced compared to suckled counterparts (22 vs. 32 cells). Also, it was observed that regression of the mammary gland was not uniform throughout all regions. Greater involution occurred in the parenchymal tissue localized just above the gland cistern compared to the tissue adjacent to the ventral body wall (Akers et al., 1990).

Observations from a study completed by Capuco et al. (1997) using prepartum Holstein cows differed to some extent from that of Akers et al. (1990). Their objectives were to observe involution, growth and differentiation of epithelial cells in mammary glands of experimental cows that were milked during the last 60-d of gestation (no dry period), and to compare the findings with mammary glands of cows that had a regular 60-d dry period. Important and significant findings were that no net loss of mammary cells (involution) and no regression of alveolar structure occurred in cows during the dry period. In addition, at 7 d prepartum the proportion of epithelial cells was greater in dry cows than in lactating cows, indicating a greater renewal of damaged or senescent cells during the dry phase. One similar observation to that observed in the study of Akers et al. (1990) was that only the lower region of the mammary gland (adjacent to gland cistern) appeared to be most sensitive to stimuli promoting renewal, not involution of epithelial cells.

The renewal of damaged cells is facilitated because of remodeling of the parenchymal tissue during the dry period. During the first 30 d of a 60-d dry period Athie et al. (1997) observed that the plasminogen and plasmin system seemed to be responsible for the remodeling. Plasminogen is the inactive zymogen of plasmin, a proteolytic enzyme that carries out the breakdown of components of the extracellular matrix that surrounds the alveoli (collagen type IV). The researchers observed that there was a decrease in plasminogen:plasmin ratio, an increase in plasmin concentration, and in the proteolytic activity of plasmin in mammary secretions as the dry period progressed. The secretion of plasminogen activators by somatic cells (macrophages) and myoepithelial cells was considered to be the cause of these physiological events.

Capuco et al. (1997) also observed that none of the epithelial cells were in a secretory stage at 35 d prepartum in mammary glands of Holstein cows that had a 60-d dry period. At 20 d prepartum, the number of cells in a secretory stage increased substantially, and by 7 d prepartum alveolar luminal area was at its maximum, reflecting the initial accumulation of colostral secretions. These changes in secretion pattern indicated that by 20 d prepartum (beginning of the prepartum transition period) there was a significant increase in mammary gland activity toward milk secretion. Oliver and Sordillo (1989) characterized this phase as phase I lactogenesis, whereas phase II began shortly before calving and was characterized by copious secretion of colostrum.

It is important to mention that the study conducted by Akers et al. (1990) utilized non-pregnant beef cows at 59 d postpartum. The Holstein cow study (Capuco et al., 1997) utilized late pregnant cows during late lactation. Contrasting results obtained may be due, in part, to breed differences, although they more likely were due to reproductive and lactational stage of the experimental cows. These observations indicated the possible involvement of hormones in regulating mammary tissue renewal during the dry period. In fact, the prepartum transition period is marked by tremendous changes in concentrations of reproductive and non-reproductive hormones that directly and/or indirectly impact mammary gland function of ruminants (Anderson et al., 1981; Neville et al., 2002).

Knowledge about lactogenesis was gained from studies that dealt with hormonal induction of lactation in dairy ruminant species (Head, 1999). One of the early studies to address this issue was conducted by Smith and Schanbacher (1973). In their experiment, various breeds of nonlactating dairy cows (n=9) and one nulliparous heifer were injected with a mixture of 17 β-estradiol (E_2) and progesterone (P_4) twice a day for 7 to 10 consecutive days. The treatments were successful in inducing lactation in 7 of the 10 animals (including the heifer) by about 19.3 d after termination of the steroid injection period. This strongly indicated that these steroid hormones were necessary to initiate mammary growth, cell differentiation and milk production.

In addition to E_2 and P_4, prolactin, corticoids and somatotropin also are required for initiation of lactation, as demonstrated in numerous studies summarized in reviews (Delouis et al., 1980; Head, 1999; Tucker, 2000). In cows, total estrogens begin to increase at 10 d prepartum to a maximum of 5 ng/mL, but concentrations decrease sharply soon after calving. At the same time, there is a progressive decrease in concentration of P_4 from 7 ng/mL to a minimum concentration (< 1.0 ng/mL) at parturition. Concentrations of prolactin, somatotropin and corticoids generally increase significantly beginning at 5-7 d before parturition, with peak concentrations around calving. Concentrations at peak were about 150, 25 and 10 ng/mL for prolactin, corticoids and somatotropin, respectively (Head, 1999).

Ovarian steroids (E_2 and P_4) are considered necessary to induce lobulo-alveolar formation in mammary tissue, mainly in heifers during the last trimester of their first pregnancy. In multiparous cows, E_2 has a mammogenic role, which is potentiated by P_4. Progesterone is a well-known inhibitor of lactogenesis, and its withdrawal during the peripartum time period is related to the initiation of lactogenesis. Prolactin has an important role in the formation of the lobulo-alveolar structure, but high concentrations at parturition are more important to induce copious milk secretion. With regard to corticoids, studies conducted with ewes have shown that injections of hydrocortisone acetate were required to stimulate appropriate lactogenic response in nulliparous animals (secretion of β-casein). In addition, corticoids potentiate action of prolactin on lactogenesis (Delouis et al., 1980). Early studies recognized that somatotropin ensured maintenance of lactation, but it was not critical for mammogenesis and lactogenesis (Peel and Bauman, 1987) because the hormone did not exert a direct effect on mammary growth or lactogenesis. This latter action likely was due the absence of the somatotropin receptor in epithelial cells (Keys and Djiane, 1988).

Recently, Sinowatz et al. (2000) found evidence of immunoreactive somatotropin receptor (irSTR) in the epithelial and stromal compartments during different stages of mammary gland development (mammogenesis, lactation and involution). The ductular epithelium showed irSTR during most stages, whereas the alveolar epithelium contained a modest amount of irSTR during pregnancy, but this increased during lactation. In dry cows, irSTR in the alveolar epithelium was very weak or negative, confirming the observations that somatotropin could not have a direct effect on mammary epithelial cells during the dry period. Effects of direct action of somatotropin in mammary cells during lactation remain to be determined.

Despite the absence of somatotropin receptor in mammary epithelial cells, somatotropin has a strong indirect effect on dry period mammogenesis through actions of insulin-like growth factor-I (IGF-I) secreted by the liver, and by other tissues that have somatotropin receptor, such as adipocytes (Thissen et al., 1994) that also are present in the mammary gland (Chilliard, 1999). There is an abundance of IGF-I receptors in mammary epithelial cells during the dry period, and it is well known that the growth factor and its binding proteins exerts significant regulation (inhibition) of mammary epithelial cell apoptosis during the dry-period (Accorsi et al., 2002; Blum and Baumrucker, 2002).

Review of previous research leads to the conclusion that a dry period is necessary for the renewal of epithelial mammary tissue, and that the hormonal events of the prepartum transition period are necessary to stimulate the tissue regeneration (remodeling). Milk stasis initiates a cascade of events in the parenchymal tissue that culminates with the replacement of damaged and senescent cells. Estrogen, P_4 and IGF-I are the most likely hormones or growth factor responsible for the stimulation of cell renewal. Prolactin, withdrawal of P_4 and corticoids are importantly responsible for lactogenesis, which is divided into phases I and II. Phase I begins about 30-14 d before calving, and phase II (colostrogenesis) begins just a few days before calving. Finally, somatotropin is not directly required for mammogenesis and lactogenesis, but IGF-I secreted under somatotropin stimulation, is an important indirect effector of somatotropin for mammogenesis and lactogenesis.

Diseases and the Transition Period

A successful transition from the dry period into lactation is achieved only when adequate metabolic changes occur. However, despite the physiological efforts by the cow to carry out these changes, some functions are often impaired, that lead to diseases. It is during the first 2 wk of lactation (postpartum transition period) that most diseases of dairy cows occur (metabolic [milk fever - MF, ketosis - KETO, displacement of abomasum - LDA], infectious [clinical mastitis - MAST] and reproductive [retained placenta – RP and metritis - MET]). In addition, some disorders that are not diagnosed during this early lactation phase, such as laminitis (LAME), have causes (etiology) that can be traced back to insults that occurred during early lactation (Erb and Gröhn, 1988; Goff and Horst, 1997).

To better understand the epidemiology of the diseases mentioned, a number of studies were conducted to identify causes, risk factors, and also to look for associations among the disorders (epidemiological parameters; Belyea et al., 1974; Roine and Saloniemi, 1978; Erb et al. 1985; Gröhn et al., 1989; Rajala-Schultz et al., 1999; Fleischer et al., 2001). All information gathered proved to be useful for the development of strategies for disease prevention and necessary, not only to promote animal well-being, but also to minimize the enormous negative economic impact that these diseases have on the dairy industry. In the US, economic losses from MF, RP, KETO, LDA, and LAME have been estimated to be $ 335.00, 285.00, 145.00, 340.00, and 302.00 per case, respectively. Overall, these losses include prophylactic or clinical treatment, lost milk production, increased culling, and cost of surgery in case of LDA (Kelton et al., 1998).

Reports of epidemiological parameters for the diseases mentioned previously were made based upon experiments that studied lactation incidence rates (LIR, number of cases per lactation divided by 100 lactations) of diseases in a specific population, or by carrying out analyses of results from many published studies to draw more broad conclusions. Using the first approach, Erb et al. (1985) conducted a study to develop and evaluate models and relationships among productive variables and disorders of the transition period using data from 784 primiparous heifers and 2,066 multiparous Holstein cows. The LIR for RP, MET, MF and MAST in heifers and cows were 3.6 and 12.1, 10.2 and 12.3, 0 and 6.5, and 6.3% and 10.5%, respectively. In another study, Gröhn et al. (1989) conducted an extensive analysis of 61,124 Finish Ayshire lactations. Among heifers and cows, the LIR and time of diagnosis during the lactation of MF, LDA, KETO, RP, MET (early) and MAST (acute) were 4.0, 0.5, 6.0, 4.4, 2.3 and 6.2 % and 1, 21, 28, 2, 16, 44 DIM, respectively.

Erb and Gröhn (1988) and Kelton et al. (1998) used analyses of results from published research to draw conclusions about disease incidence. In the first study, information was gathered from 70 epidemiological (observational) studies conducted with Holstein-Friesians and Finnish Ayrshire dairy cows. For these studies, the LIR for MF, LDA and KETO observed varied from 1.2 to 14.1, 1.22 to 2.5 and 1.1 to 9.2%, respectively. Diagnosis of MF, LDA and KETO occurred between 0-15, 0-13, and 21-35 DIM, respectively. In the second study (Kelton et al., 1998), data from 195 research publications that appeared between 1970 and 1996 for various dairy breeds were used to draw conclusions about LIR. The overall LIR ranges for MF, RP, MET, KETO, LDA, LAME and MAST were 0.03 to 22.3, 1.3 to 39.2, 2.2 to 37.3, 1.3 to 18.3, 0.3 to 6.3, 1.8 to 30, and 1.7 to 54.6%, respectively. The median LIR for the diseases were 6.5, 8.6, 10.1, 4.8, 1.7, 7.0, and 14.2%, respectively. The information presented indicated that LIR for all diseases were similar, and that most diseases were diagnosed during the early postpartum period (0-50 DIM).

In addition to incidence rates, some studies evaluated impact of these diseases in the reduction in milk production. In an experiment conducted using 500 Holstein cows (Deluyker et al., 1994), those with Metritis, MAST, and KETO had MY reductions of 266, 281, and 253 kg during the first 120 DIM, respectively. Displaced abomasum caused a reduction of 402 kg during the first 49 DIM. An interesting observation was that decrease in MY production occurred even before diagnosis of some diseases, such as MET. The LIR and diagnosis of MET, MAST, KETO and DA were 5.6, 26.1, 18.3 and

2.0%, and 24, 26, 21 and 21 DIM, respectively. In a more comprehensive study, Rajala-Schultz (1999) analyzed the effects of disease and parity (adjustment) on milk yield loss of 22,242 Finnish-Ayrshire cows. For primiparous and 2, 3 and 4+ parity cows, MY losses due to MF were 47.6, 37.8, 79.8 and 33.6 kg during the first 56 d after the diagnosis. For KET, 126.0, 126.0, 67.2 and 535.4 kg of milk were lost between 28 d before and 42 d after diagnosis (70 d time frame). The LIR for MF and KETO for 1, 2, 3 and 4+ parity cows were 0.2, 1.1, 5.7, 16.7%, and 2.5, 3.2, 4.2 and 4.1%, respectively. The average MY loss for KETO reported by Rajala-Schultz (1999) was 213.7 kg, similar to the 253.4 kg reported by Deluyker et al. (1994). In older cows, greater MY loss probably was due to their greater capacity for milk production as they grow older (Head, 2000). The LIR of both studies reported were in the range previously mentioned.

Others used different approaches to evaluate associations between MY and LIR of diseases. In a study conducted using 2,197 lactations of Holstein-Friesian cows, Fleischer et al. (2001) hypothesized that MY could be the cause of disease. Indeed, trends of correlations between previous lactation MY and RP, MAST and MF were observed ($P \leq 0.10$). In addition, there were significant ($P \leq 0.05$) negative correlations between current lactation MY and claw diseases (LAME) or cystic ovaries (CYST). The estimated probability of appearance (EPA) for all diseases increased as lactation MY increased from 6,000 to 12,000 kg. The 6,000 and 12,000 kg MY EPA for RP, MAST, MF, LAME and CYST were 6.4 and 17%, 17.9 and 44.2%, 4.0 and 13.2%, 16.2 and 32.2 %, and 9.0 and 27.5 %, respectively.

In addition to the previously described associations, others have evaluated possible relationships among diseases. One of the first researchers to indicate possible correlations was Morrow (1976) when characterizing fat cow syndrome. This was described previously in the BCS section of this review (page 17). Cows with this syndrome had ketonuria (clinical sign of ketosis) that usually was associated with MF, DA, digestive problems, RP, MET, MAST and salmonelosis (diarrheas). Following these observations, others (Curtis et al., 1983; Thompson et al., 1983; Markusfeld, 1984, 1985; Gerloff et al., 1986; Correa et al., 1990; Kaneene et al., 1997; Melendez et al., 2003) evaluated possible correlations among diseases, as well as the correlation between etiological factors of one disease with other disorders. In most studies, the odds ratio (OR, the antilogarithm of the coefficient for an independent variable in a logistic regression) was used as an approximate measure of relative risk. An OR greater or smaller than 1 implied increased or decreased risk of contracting a disease with increasing value of independent variable (cause), respectively (Gröhn et al., 1989).

Using the approach described above to establish associations between diseases, Curtis et al. (1983) evaluated associations between MF and 7 periparturient disorders (dystocia, RP, MET, LDA, LAME, KETO and MAST) in 33 Holstein dairy herds (2,190 cows). Significant ($P<0.01$) associations between MF and dystocia, RFM, KETO and MAST were detected. The OR were 6.5, 3.2, 8.9 and 8.1, meaning that cows that had MF were 6.5, 3.2, 8.9 and 8.1 times more likely to have each of the other disorders. In a subsequent study, Markusfeld (1984) observed that Israeli-Friesian cows with long dry period, and that had RP or MET, were 3.0 and 3.1 times more likely to develop ketosis than healthy cows. In addition, among all ketotic cows in the population (LIR 18%), 82.4% had at least one concurrent disease (RP, MET, DA). The long dry period was observed to be a predisposing factor because cows (usually low-yielders) became overconditioned during the long period dry. This indicated there was a relationship between management and disease incidence.

Gerloff et al. (1986) observed that higher culling rate of postpartum dairy cows was associated with severe hepatic lipidosis (HL) caused by a greater loss of BCS during the early postpartum period. For severe and mild HL cows, BCS at calving was similar, but BCS loss during early postpartum period was 1.17 and 0.72 pt., respectively. As a consequence of HL, serum NEFA and liver TAG (wet basis) concentrations at 3 wk postpartum and culling rate were ~400 and 750 μEq/L, 2 and 11%, and 15 and 42%, for mild and severe HL cows, respectively. Although there only were trends of significance between culling rates, they did indicate the possibility of existence of this association. In another study, Kaneene et al. (1997) also observed trends of the possible relation of metabolic events associated with energy insufficiency (increased fat mobilization and serum lipoprotein metabolism) with increased risk of MET and RP.

The results of all experiments described are just examples of some relationships among milk production and periparturient disorders. The wealth of information in some studies (large data sets), allows researchers to draw broad and significant conclusions about the relationships found. To better understand these, path analysis (regression models) models were proposed. An example of a path analysis is in Figure 3. It represents significant relationships (OR) among metabolic disorders obtained from a study using 61,124 Finnish-Ayrshire cows (Gröhn et al., 1989). A disease indicated by the arrow means that it is a likely outcome when the preceding disorder (risk factor, at the beginning of the arrow) occurs. In the model presented, it is important to mention that most of the 10 disorders lead to ketosis and abomasal disorder, and also that ketosis is a predisposing factor for some diseases.

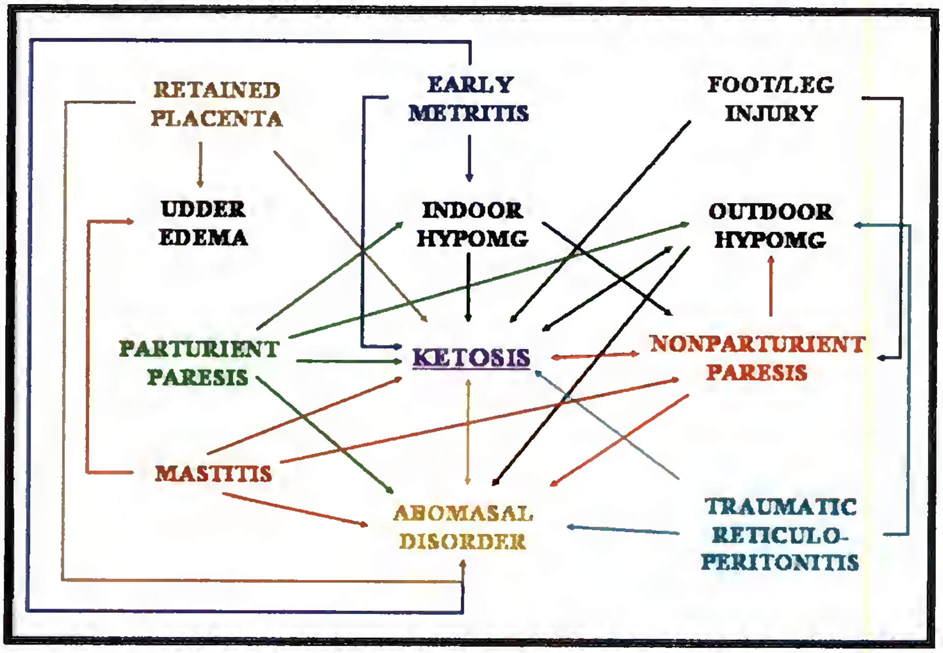

Figure 3. Relationships among metabolic disorders. Hypomg stands for hypomagnesemia (Adapted from Gröhn et al., 1989).

It is during or following the transition period of dairy cows that most metabolic, reproductive and infectious diseases occur. Diseases included are MF, KETO, DA, MAST, RP and MET. Results of many experiments indicated that these diseases occur to a similar extent in various dairy populations. Another important observation is that the likely outcome of any disease is ketosis. This underlines the importance of improving the metabolic adaptations involving liver and fat metabolism during the transition period. This would require appropriate dietary management during the prepartum and postpartum periods, through strict control of BCS status, and through the maintenance of adequate DMI of a well-balanced ration.

Recombinant Bovine Somatotropin (bST) and Lactation in Dairy cows

The scientific and management information arising from dairy research involving somatotropin (ST) has progressed far beyond understanding the role of somatotropin in the metabolic adaptations seen during the transition period. Since the discovery that "growth hormone" or somatotropin (the greek derivative) was the substance in crude extract of bovine pituitary glands responsible for stimulating the growth of rats, and for increasing milk yield of goats and hundreds of dairy cows in the late 1920s, the galactopoietic potential of somatotropin gained more attention (Etherton and Bauman, 1998). Through the late 1970s research studies using bST were limited because adequate amounts of hormone required for large scale study could not be provided from the only source at that time (slaughtered animals). However, the advent of recombinant DNA technology in the early 1980s enabled an impressive number of researchers to investigate the galactopietic effects of somatotropin in dairy cows, once significant quantities of the hormone could be produced *in vitro* (recombinant bST, rbST; Peel and Bauman, 1987; Bauman and Vernon, 1993).

Somatotropin is a large protein hormone (190 or 191 amino acids; mol. wt. 22,000 Daltons) produced by the acidophilic cells (somatotrophs) in the anterior pituitary. Secretion of somatotropin is regulated by two hypothalamic peptides that act to stimulate (growth hormone-releasing factor, GRF) or inhibit (somatostatin) release of somatotropin (Etherton and Bauman, 1998). The hormone has four major variants in cattle. These arise from the combination of two possible N-terminal amino acids (alanine or phenylalanine) and two possible amino acids at position 127 of somatotropin (leucine or valine). In the dairy breeds Brown Swiss, Holstein, Guernsey, Ayrshire and Jersey, the frequencies of leucine-127 and valine-127 alleles detected were 1.0 and 0, .93 and .07, .92 and .08, .79 and .21, .56 and .44, respectively (Lucy et al., 1993). Valine-127 variant is known to elicit greater increase in milk yield than the leucine variant (Etherton and Bauman, 1998). At the cellular level, somatotropin has a unique effect compared to other protein hormones. The somatotropin membrane receptor (cytokine-type of transmembrane receptor) can recognize two different sites of a somatotropin molecule, allowing one molecule to bind two receptors at the same time, forming a dimer (Figure 4). After binding and dimerization, a cascade of intracellular signal transduction pathways is initiated (PKC pathway), ending with the binding of signaling molecules in the target genes (Maharajan and Maharajan et al., 1993).

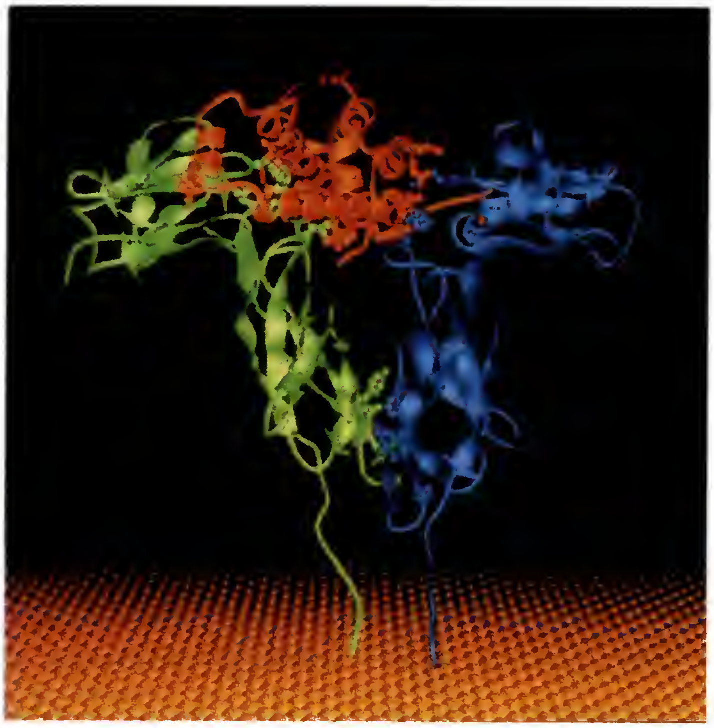

Figure 4. Backbone structure of the hGH-(hGHbp)$_2$ complex. The hormone, receptor I and receptor II are shown as red, green and blue β strands and loops, respectively (De Vos, A. M., M. Ultsch and A. A. Kossiakoff. 1992. Science 255(5042): 306-312. © AAAS).

Investigations of rbST effects in dairy cows began with short-term studies (5-21d) conducted during the early 1980s. These evaluated daily injections of the hormone, and results agreed that gradual increase in milk yield occurred after a few days of rbST treatment. Maximum production was reached during the first week after the first injection. When treatment was terminated, milk yield gradually returned to pretreatment levels over a similar period of time. No increase in feed intake was observed in these studies. Increases in MY varied from 5 to 40% (Peel and Bauman, 1987; Bachman et al., 1999). The variations in response occurred due to duration and dose, lactation stage, management conditions, and other unidentified factors.

One of the first studies to address the effects of long-term use of rbST on production responses of dairy cows was conducted by Bauman et al. (1985). In their experiment, 30 multiparous Holstein cows supplemented with 0, 13.5, 27 and 40.5 mg/d of methionyl bovine somatotropin (MBS) or with 27 mg/d of pituitary bovine somatotropin (PBS) produced 27.5, 34.4, 38.0, 39.4 and 32.5 kg/d of FCM during the period from 84 ± 10 through 188 DIM. Increases in milk yield were 23.3 to 41.2% over the dose ranges tested. Increases in net energy intake occurred 5-9 wk after the initiation of treatments. The average energy balance across the treatment period for all somatotropin supplemented groups was positive and sufficient to replenish body stores that had been mobilized and used during early lactation. In addition, treatment with somatotropin showed a trend to increase feed efficiency to produce milk (ratio of FCM yield/NE Mcal). Increases in milk yield with different bST doses also were reported by many researchers including Hansen et al. (1994). In their study, supplementation with bST began at 28 to 35 d postpartum, and they observed increases of 10.5 and 9.0% of

3.5% FCM in multiparous cows and primiparous heifers injected with 5.15 and 16.5 mg/d of bST during a whole lactation, respectively, compared to non-supplemented controls.

Bachman et al. (1999) summarized results of a large number of long-term field studies (15) conducted to evaluate the effects of daily or bi-weekly (14 d, sustained release formula) injections of rbST on production responses of dairy cows. The number of cows, dose used, duration of experiments, and time of treatment initiation ranged from 6-63, 20.6-50.0 mg/d, 70-266 d and 28-56 d postpartum, respectively. Average control MY (kg/d) and DMI (kg/d) ranged from 19.8-29.8 and 15.5-23.7, respectively. Average increase in MY (kg/d) and DMI (kg/d) in treated cows ranged from 2.2-10.1 and 0-3.7. The average increase in MY was from 9.2 to 36.2 % in rbST treated cows. Although significant increases in DMI were observed, in 13 of the 15 studies the cows were in negative EB balance during the period of supplementation.

Subsequently, Bauman et al. (1999) examined production responses in northeast commercial dairy herds after POSILAC® [prolonged-release formulation of the rbST (500 mg/14d; 35.7 mg/d) made by Monsanto Co.] was approved for commercialization in the US in 1994. They conducted an extensive study using 200,000 individual DHI lactation records obtained from herds that utilized or did not utilize bST supplementation. Data were collected over an 8-yr period (4-yr pre-approval of bST and 4-yr post-approval commercial availability of POSILAC®). During the 4-yr post-approval period, dairy cows in bST herds on average produced more milk (2.93 kg/d), fat (88 g/d), protein (100 g/d) and had slightly higher SCC in milk than control non-supplemented cows in the same herd. No effect on milk composition was observed due to bST. Milk response to bST was minimal during the first 60 d post-injection, and then gradually increased until reaching a plateau that was maintained over the last one-half of the lactation. In addition, lactation persistency was greater for bST injected cows. No adverse effects of bST on health were observed, because herd-life of animals was not altered by bST treatment.

The increases in milk production observed in bST-supplemented cows resulted from a favorable adaptation of the diverse physiological processes that support milk production, and involve the metabolism of all nutrient classes. These adaptations are directly and indirectly mediated by somatotropin. Because adipocytes and hepatocytes have somatotropin receptors, these cells are direct targets of the hormone. Indirect actions in cells are mediated by somatotropin-dependent insulin-like growth factors (IGF-I, IGF-II and the IGF binding proteins [IGFBPs; I-VI]), which exert most somatotropin actions in the mammary gland due to the extensive presence of specific IGF-I receptors in the organ and in other body tissues that give support to milk production, such as muscles (Bauman, 1999).

In a 1993 review, Bauman and Vernon summarized the effects of bST on specific tissues and physiological adaptations in lactating cows. In the mammary gland, somatotropin (mainly through IGF-I) increased blood flow and maintenance/activity of secretory cells. These events led to increased uptake of nutrients for milk synthesis, which was substantially enhanced and had normal composition. At the whole body level, somatotropin increased glucose availability for lactose (milk) synthesis by decreasing muscle use and overall body oxidation of glucose, and by increasing the rates of liver gluconeogenesis. This latter metabolic event occurs because of the inability of insulin to inhibit hepatic gluconeogenesis when somatotropin is supplemented and concentrations are greater, and also by the increase in voluntary feed intake to match the need of the mammary gland and other organs and tissues for glucose that has been observed following long-term use of bST. In addition, somatotropin increases immune response, circulating IGF-I, IGF binding protein 3 (IGFBP-3, which acts as one of the regulators of the biological action of IGF-I; Jones and Clemmons, 1995), NEFA oxidation, if cows are in negative energy balance, energy expenditure/cardiac output consistent with increase in milk yield, productive efficiency, and also decreases AA oxidation. With regard to the adipose tissue, the adipocyte is a major target for somatotropin action. It regulates the biochemical pathways depicted in Figure 1. Under somatotropin supplementation, dairy cows in positive energy balance have decreased lipogenesis, because the hormone reduces the lipogenic action of insulin and adenosine, an autocrine/paracrine factor. If dairy cows are in negative energy balance under somatotropin supplementation, there is an increase in basal lipolytic rate due to enhanced sensitivity of adipocytes to catecholamines (Jaster and Wegner, 1981; Vernon and Pond, 1997; Rukkwamsuk et al., 1999).

In a comparison to the genetically superior cow (GS cows), bST supplemented cows (bST cows) did not differ for most of the variables evaluated (Peel and Bauman, 1987). Digestibility of feed, maintenance, partial efficiency of milk synthesis, and overall efficiency (kg milk/kg feed) did not differ between the two groups. However, GS cows better utilized their body reserves during early lactation, and they also had higher feed intake than bST cows. In bST cows, increased mobilization of body reserves to provide needed nutrients and increased feed intake occurred 5 wk after beginning of bST supplementation. The mammary gland of GS cows had greater quantities of secretory tissue, but activity per secretory cell was not known. In bST-supplemented cows there is an increase in synthetic rate/activity per cell in the mammary gland, but not in number of secretory cells (Capuco et al., 2001). However, in both GS and bST-supplemented groups of cows there is a need for improved management to optimize reproductive performance, and to reduce the calving interval, which otherwise is increased. These comparisons indicated that any dairy cow with good productive potential can become more like a genetically superior animal by supplementing it with bST.

The advent of recombinant DNA technology during the early 1980s also has led to the greatest rate of advance in the field of lactation biology. Recombinant bST allowed a great number of researchers to adequately explore overall potential to increase milk production of dairy animals, and also to understand the physiological mechanisms that elicited such responses. The increase in mammary gland activity due to the direct actions of IGF-I and IGFBPs, changes in adipose tissue and liver function due to direct actions of somatotropin, and indirect actions on overall body metabolism are key events that led to increased productive performance. These latter findings also improved knowledge about the possible roles of somatotropin in metabolic adaptations observed during the transition period.

Strategies to Improve Metabolic Adaptations during the Transition Period

Among all metabolic adaptations seen during the peripartum phase, those related to lipid metabolism appear to be the most important. The modern dairy cow relies heavily upon the mobilization of lipids that then are used as an energy source for peripheral tissues (ketone bodies), and also as a precursor for synthesis of milk fat by the mammary gland (NEFAs) during early lactation. Increases in lipid metabolism push hepatic functions to the limit, and any imbalance in the synchrony of metabolic events can lead to a greater increase in fat mobilization, a change that can lead to disease (ketosis). Attempts to improve overall functionality during the transition period should focus on increasing the availability of energy sources for liver gluconeogenesis, to minimize mobilization of lipids, and to improve export of fat from the liver (Grummer, 1993).

In a review article, Grummer (1995) summarized and evaluated some findings that could be used to improve metabolic adaptations during the transition period. One of the first observations was that DMI, as a percentage of BW, at d 21 postpartum was positively correlated with DMI at d 1 prepartum. One likely explanation for this is that overconditioned cows during the prepartum had a poor appetite postpartum (high body availability of energy precursors [fat]). Therefore, management strategies to avoid overconditioning of cows during the dry period should be used. Another approach would be to offer diets differing in energy (NE_L) and CP contents during the prepartum period. In general, observations were that cows fed diets with increased nutrient concentrations (increased proportion of concentrate in the diet) had increased DMI during the time period from d 21 to 7 prepartum. As expected, these differences decreased and were lost as parturition approached. Furthermore, the higher intake of nutrients during that 2 wk period prepartum caused a more severe decrease in DMI during the final week before calving.

Despite the potential negative effects of DMI, the benefits of feeding high energy diets may offer greater benefits than simply providing more calories. For example, the increase in prepartum dietary energy content initiates the appropriate adaptations in the rumen to the postpartum diet, which is high in overall and specific nutrient content to support milk production (National Research Council [NRC], 2001). Changes include those in the microflora population and an increase in length of papillae that, in times needed, increase the production and absorption of gluconeogenic VFA (proprionate) compared to non-adapted cows (Goff and Horst, 1997). Increased gluconeogenesis minimizes the depletion of hepatic glycogen stores, increases concentration of glucose and insulin in serum, decreases serum concentrations of NEFA and TAG deposition in liver, and potentially reduces ketogenesis (Grummer, 1993). In fact, these improvements in metabolic adaptations were observed by Doepel et al. (2002). In their experiment, Holstein cows fed a high energy (HE) diet (1.65 vs. 1.30 Mcal/kg of NE_L) during the prepartum transition period had increased postpartum DMI and improved energy balance during both periods. In addition, HE diet reduced plasma concentrations of NEFA at calving and reduced hepatic TAG content.

Other methods that could be used to increase quantities of gluconeogenic precursors include oral supplementation of propylene glycol (PG) as a drench (Grummer, 1995) or including ionophores in the TMR (Vallimont et al., 2001). The use of these compounds during the transition period increased concentrations of glucose in blood and decreased concentrations of NEFA.

Another strategy that could be used to improve extent and timing of metabolic adaptations in cows during the transition period is the supplementation of recombinant bovine somatotropin (bST). The overall effects of bST on the metabolism of lactating dairy cows (Bauman, 1999) could be beneficial to the transition dairy cow. During the early 1990s, researchers supplemented dairy cows with bST before calving or during the early postpartum period (transition period) to evaluate effects on MY (Bachman et al., 1992; Santos et al., 1999; Putnam et al, 1999), on improvement of recovery of diseased animals with fat cow syndrome (Maisey et al., 1993; Laven and Andrews, 1998), and on mineral metabolism (Law et al., 1994; Eppard et al., 1996).

Results of previous studies were variable. For example, Bachman et al. (1992) did not observe any positive or negative effects of bST-supplementation (25 mg/d) from d 21 to 7 prepartum on MY of cows during the subsequent lactation. However, Santos et al. (1999) did observe significant effects of bST (500 mg/14 d) on daily MY of Holstein cows injected from 5 through 45 DIM. Despite an increase in MY (about 10%), DMI was slightly less in bST-supplemented cows. Results indicated there was increased efficiency of nutrient utilization to produce milk in the bST-supplemented cows. Putnam et al. (1999) observed similar results with regard to production responses. In their study, Holstein cows supplemented with bST (500 mg/14 d) from d 28 prepartum through d 42 postpartum had substantial increases in MY. Cows supplemented with bST produced 3.3 kg/d more milk than control cows through 42 DIM, but DMI was slightly higher in supplemented cows, in contrast to results of Putnam et al. (1999).

Studies of Maisey et al. (1993), Laven and Andrews (1998), and Eppard et al. (1996) obtained contrasting results. In the first experiment (Maisey et al., 1993), cows with fatty liver syndrome that were supplemented with 640 mg of bST showed no increase of plasma concentrations of glucose or B-HBA, indicating that bST did not lead to clinical ketosis. In the study by Laven and Andrews (1998), Jersey cows with signs of fatty liver syndrome and supplemented with 640 mg of bST had greater plasma concentrations of NEFA and B-HBA than diseased non-treated cows. In the third study (Eppard et al., 1996), transition Jersey cows injected with 500 mg bST/14 d from 28 d prepartum through 14 d postpartum had an increase in duration but not in the incidence of clinical ketosis. The bST led to greater concentrations of B-HBA in blood of supplemented cows than non-supplemented cows.

The research of Maisey et al. (1993), Laven and Andrews (1998), and Eppard et al. (1996) indicated that bST-supplementation caused both positive or negative effects on dairy cows during the transition period and early lactation. However, it is important to mention that in each study they supplemented high doses of bST (22.8-35.7 mg/d). Others have explored the potential of using low doses of bST (5-15 mg/d) during the postpartum period (Stanisiewski et al., 1992) or during the dry period (Simmons et al., 1994) to evaluate the effects on production, overall metabolism, health and reproduction. In the first study (Stanisiewski et al., 1992), multiparous Holstein cows injected with 5 or 14 mg of bST from 0-60 DIM and from 61-130 DIM had significant increases in FCM/d (1.2 and 1.3 kg for 5 and 14 mg/d of bST, respectively) compared to non-supplemented control cows. From 61-130 DIM, increases were 2.7 to 4.1 kg/d. Groups of cows supplemented with low doses of bST had increased pregnancy rates and first conception rates, and no adverse effects on health were observed. In the second study (Simmons et al., 1994), Holstein cows were supplemented with 0, 5 or 14 mg/d of bST during the last 46 ± 6 d before expected parturition. Supplementation with low doses of bST significantly increased concentrations of somatotropin in blood (6.5 vs. 22.7 ng/mL), better maintained IGF-I concentrations as parturition approached, and did not impact serum concentrations of NEFA during the treatment period. These studies indicated that supplementing low doses of bST may positively impact production, reproduction and health of transition cows, without showing the adverse effects on metabolic adaptations that were observed when full doses were used during similar time period.

In subsequent studies (Garcia-Gavidia et al., 2000; Gulay et al., 2000), multiparous Holstein cows were supplemented with bST at 0, 5.1, 10.2 and 15.3 mg/d during the prepartum and postpartum periods. Objectives were to evaluate the effects on productive and metabolic responses. In both experiments, injections began at 3 wk prepartum and extended through ~65 DIM. In the study of Garcia-Gavidia (2000), groups of control (non-supplemented) and bST-supplemented cows (5.1 mg/d of bST) were fed postpartum diets containing 0 or 15% whole cottonseeds. During the prepartum, bST-supplementation increased concentrations of glucose, but no effects were observed on mean concentrations of insulin or IGF-I in plasma. The prepartum DMI of bST-supplemented cows was slightly greater than non-supplemented cows. In addition, no significant effects of bST, diet or their interactions were observed on any variables during the first ~65 DIM. The milk production was numerically but not significantly greater in bST-supplemented cows (39.27 vs. 37.69 kg/d, an increase of 4.2%).

In the study conducted by Gulay et al. (2000), treatments included prepartum and postpartum supplementation with various amounts of bST (0, 5.1, 10.2 and 15.3 mg/d; groups I to IV, respectively) that began at the same time prepartum as in study of Garcia-Gavidia (2000). Effects of bST-supplementation on DMI, BCS, BW, MY, metabolic hormones and metabolites were evaluated. During prepartum, the two greater amounts of bST (10.2 and 15.3 mg/d) caused increases in plasma concentrations of somatotropin, but only the greatest amount (15.3 mg/d) increased plasma concentration of IGF-I. During the postpartum, the 10.2 and 15.3 mg/d amounts of bST caused increases in plasma concentrations of somatotropin, IGF-I, T_3 and numerically greater MY (30.93, 31.23, 32.03 and 34.93 kg/d for groups I to IV, respectively). However, for BCS, cows in groups

III and IV better maintained BCS than cows in groups I and II. No adverse effects of bST-supplementation were observed during the experimental period.

Subsequently, Gulay et al. (2000, 2003b) evaluated the effects of bST supplemented at 10.2 mg/d during the prepartum and postpartum transition period on DMI, BW, BCS, MY and plasma concentrations of IGF-I, insulin and NEFA of Holstein cows. Supplementation began about 21 d prepartum and was extended through 42 DIM. No effects of bST supplementation were detected on DMI, BW and BCS during the prepartum and postpartum periods. Supplemented cows had numerically but not statistically greater MY (FCM 3.5%) than non-supplemented cows. During prepartum, no differences were detected for mean concentrations of glucose or NEFA, and not for somatotropin, as was expected. During the postpartum, no differences were detected for mean concentration of insulin, glucose or NEFA. Plasma concentrations of somatotropin and IGF-I were greater in bST-supplemented cows than non-supplemented cows, as expected.

The numerically greater increases in MY observed for bST-supplemented cows in these studies, without an adverse effect on postpartum DMI, BCS, or concentration of NEFA in plasma, indicated that daily amounts of bST had a significant positive impact on transition dairy cows. Because milk production is highly associated with health rates, as mentioned in a previous section, bST may also have played a role in improving the metabolic adaptations to avoid disorders, mainly related to liver metabolism. Somatotropin is a metabolic hormone that has an important role in regulating some of the adaptations that involve adipose tissue metabolism and liver function. Therefore, use of bST-supplementation has the potential of stimulating desirable positive changes.

However, to date, no study has been carried out to critically evaluate the effects of supplementing low doses of bST (10.2 mg/d) during the transition period, or only during the prepartum or during the early lactation on changes in metabolic adaptations involving overall metabolism, on liver metabolism of carbohydrates and lipids, on health, and any possible effects of these changes on productive responses of multiparous Holstein cows.

CHAPTER 3
MATERIALS AND METHODS

All cows used in this experiment were housed at the Dairy Research Unit (DRU) of the Department of Animal Sciences of the University of Florida, Hague, FL where the experiment was conducted. Treatments, measurements and sample collections began in August 2001 and ended August 2002. This experiment was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Florida.

Animals

One hundred and three multiparous Holstein cows were assigned randomly to the experiment when they were about 21 d before expected parturition. Cows were assigned to four treatment groups (I to IV) as they become available to enter trial during the Summer of 2001. The number of previous lactations of experimental cows at the time they were assigned ranged from 1 to 5. Average number of previous lactations for each of the four groups was 2.32, 1.84, 2.16 and 2.16 for groups I, II, III and IV, respectively. The actual days that cows assigned to the four groups spent during the prepartum period were 20, 19, 19 and 19 ± 1.46 d, respectively. The body weight (BW) and body condition score (BCS) of the cows at the time that they were assigned to treatment ranged from 598 to 889 kg, and 2.5 to 5.0 pt., respectively. The average BW and BCS were 735 kg and 3.41 pt. among all cows, and neither measures differed among TRT groups (P=0.8881 for

BW and P=0.5201 for BCS). After calving, all cows remained on the experiment through 150 DIM.

Treatments

Experimental design. Cows were assigned randomly to a 2x2 arrangement of treatments based upon period of bST supplementation during the transition period and/or early lactation. The bST used was prepared as an oil emulsion (500 mg/1.4 mL) that is marketed under the name POSILAC® for enhancement of lactation (Monsanto Co., St. Louis, MO). The amount of bST supplemented was 0.4 mL/14 d; 1/3 of the bST dose (~1.2 mL/14 d) recommended for the enhancement of lactation. This quantity provided approximately 10.2 mg/d of bST for the 2-wk injection time periods. Prepartum injections began at about 21 d (3 wk) before parturition and were extended through calving day, a total of 2 injections. Postpartum injections began approximately 24 h after calving (regardless of time of last injection) and extended through 70 DIM, a total of 5 injections. The last injection was at 56-58 DIM. The arrangements of treatments and cow numbers per TRT group follows:

Treatment I (Control)	- 26 cows	No bST Prepartum, No bST Postpartum
Treatment II (Post)	- 25 cows	No bST Prepartum, bST Postpartum
Treatment III (Pre)	- 27 cows	bST Prepartum, No bST Postpartum
Treatment IV (Pre/Post)	- 25 cows	bST Prepartum, bST Postpartum

Injections were in the left or right ischiorectal fossa and were administered after blood collection, but prior to a.m. feeding and milking. After 63 DIM, all cows, including the controls, were supplemented with a full dose of bST (500 mg/14d) during the remainder of their lactation, according to management practice at the DRU.

Feeding. During the experimental period, prepartum and postpartum cows of all treatment groups were managed and fed in the same calving pen and fresh cow free-stall barn, respectively. Diets were fed *ad libitum* to allow 5-10% daily feed refusals. Prepartum cows were fed once daily between 10:00-12:00h, and postpartum cows were fed twice a day between 10:00-1200h and again between 16:00-18:00h. Feed adjustments were made daily to allow desired feed refusals. Prepartum and postpartum diets were prepared following National Research Council (NRC, 2001) recommendations for both animal classes, and were anionic close-up diet (CUD) and TMR fresh cow diet (cationic), respectively. Diet composition and formulations are in Table 1. Diets were prepared as total mixed rations (TMR) and were group fed from a mixer wagon. No individual records of feed consumption were made through the experimental period, except group intakes were estimated daily by the feeding crew so amount offered exceeded amount consumed.

Body Weight and Body Condition Score

Body weight (BW) and body condition scores [BCS; 1 (thin, underconditioned) and 5 (fat, overconditioned); Edmonson et al.,1989] were recorded during the experiment. Each cow was weighed and BCS was estimated at time of group assignment (~ 21 d prepartum) and weekly on the same day each week (Friday) before a.m. feeding or milking (8:00-12:00h) through 70 d postpartum. Thereafter, BW and BCS were estimated biweekly until cows completed the experiment (~150 DIM).

Colostrum and Milk Samples

A sample of colostrum was collected from all cows at first milking (n=103), and samples were analyzed for quality using a commercial hygrometer (Colostrometer,

Table 1. Dry matter concentrations and Chemical Composition of Anionic close-up diet (CUD) and Fresh-Cow TMR fed to Holstein Cows[1].

Item	Anionic CUD[2]	Fresh-Cow TMR[3]
Ingredient	----------DM basis----------	
Corn Silage	44.2	21.7
Sorghum Silage	---	8.3
Alfalfa Hay	---	7.3
Cottonseed Hulls	8.8	5.5
Whole Cottonseeds	6.7	7.7
Citrus Pulp	8.5	7.7
Corn Meal	13.0	20.1
Molasses	---	2.6
Soy Plus[4]	2.2	6.5
Soybean Meal	7.0	9.4
Springer Minerals	7.4	---
Lactating Cow Minerals	---	3.3
White salt	0.3	---
Zinc Chloride	1.2	---
Magnesium Sulfate	0.7	---
Nutrient	Percentage[5]	
DM	49.41	56.66
CP	13.80	17.45
Sol CP[6]	29.42	30.51
NDF	37.42	34.74
ADF	24.49	22.36
NSC	36.12	39.88
EE[7]	4.18	4.56
TDN(%)	66.60	72.75
NE_L (Mcal/kg)	1.47	1.60
Ca^{+2}	1.66	0.65
P^+	0.34	0.38
Na^+	0.21	0.30
K^+	1.02	1.33
Cl^-	1.09	0.23
S^{-2}	0.40	0.18
Calculated DCAD[8]	-5.78	+38.75

[1] Values expected for diets formulated (National Research Council [NRC], 2001) on dry matter basis. [2] 53:47 forage:concentrate. [3] 43:57 forage:concentrate. [4] Trademark of West Central, Ralton, IA. [5] DM Basis. [6] Soluble protein as percentage of the CP. [7] Ether extract. [8] Meq (Na + K) – (Cl + S)/100 g of diet DM.

Nasco, Fort Atkinson, WI). Values obtained were recorded as mg of immunoglobulins/mL of colostrum. Milk samples also were collected bi-weekly from all cows (n=103) during three consecutive milkings (08:30, 15:00 and 01:30h) on the same day of the week for analyses of milk constituents during the first 10 wk of lactation.

Samples (50 mL) were analyzed for fat, protein and SCC contents at Southeast Milk Laboratory, Inc. (Belleview, FL). Milk yield was recorded at each daily milking from 3 d after parturition through 150 DIM.

Blood Collection, Handling and Storage

Blood samples were collected from the tail vein of all cows (n=103) three times a week before a.m. feeding or milking (06:30-10:00 h) through 70 DIM. Prepartum and postpartum cows were bled in a holding area walk through or in the free-stall barn, respectively, after elevating the tail without any other restraint. For blood collection, Vacutainer® brand needles (2.54 cm; 20 gauge) and tubes containing sodium heparin were used (10 x 100 mm blood collection tubes, Becton-Dickinson, Fairlawn, NJ). Blood samples were placed in an ice-bath immediately after collection and processed within 2 h. The order in which cows were sampled on a given day was random and differed from bleeding to bleeding. After sampling, prepartum cows were released to the dry lot, and postpartum cows were milked and then returned to the free-stall barn.

All samples of blood were centrifuged at 3,000 RPM at 5° C for 30 min (RC-3B refrigerated centrifuge, H 600A rotor, Sorvall Instruments, Wilmington, DE) to separate plasma. Plasma from each sample was aliquoted into labeled 5 mL (12x75 mm) polypropylene tubes (Sarstedt Inc., Newton, NJ), capped, and frozen at -20° C until analyzed. The plasma samples were used for analysis of insulin, somatotropin (ST), insulin-like growth factor 1 (IGF-I), calcium (Ca), glucose, non-esterified fatty acids (NEFAs) and β-hydroxybutyrate (B-HBA).

Liver Biopsies, Handling and Storage

Liver biopsies were conducted in a subset of 8, 10, 10 and 11 cows randomly selected from TRT groups I, II, III and IV, respectively. For each cow in the four subsets, puncture biopsies were conducted at about 21 d prepartum, +2, +14 and +28 d postpartum, a total of 4 biopsies per cow, as described by Hughes (1962). The procedure was conducted with cows in a restraint chute as follows. An 8x8 cm area in the medium-dorsal region of the 9, 10 or 11th intercostal space of the right side of the cow was clipped and cleaned with Betadine Surgical Scrub (10% povidone iodine, 1% glycerin, 0.25% igepal, and 88.75 % purified H_2O; Purdue Manufacturing Company, Norwalk, CT) and 70% Alcohol (Dal-Vet Phamaceudicals, Savannah, MO). The skin region to be punctured was anesthetized subcutaneously and intra-muscularly with $\cong 5$ mL of Lidocaine Hydrochloride with Epinephrine (1.0-2.0 % lidocaine hydrochloride, 0.001% L-epinephrine and purified H_2O, Elkins-Sinn, INC., Cherry Hill, NJ). Following anesthesia, a 2.5 cm incision was made in the skin with an Axicut® Sterile Disposible Scapel (Dynarex Corporation, Brewster, NY), and the liver was punctured with a custom-made biopsy needle (30 cm long by 0.6 cm wide). The liver obtained from the puncture (3-5 g) was rinsed with 0.9% saline solution, blotted dry on filter paper, placed in a cryogenic vial (Fisher Scientific, Pittsburgh, PA, R#05-669-64) and submerged in liquid nitrogen (-192 °C) until transferred to a -80 °C freezer (Bio Freezer, Mallinckrodt, INC., Marietta, OH). Following puncture, the incision was sutured using a skin stapler (3M™ Precise™ PGX Disposable Skin Stapler, St. Paul, MN), and treated once with

Nitrofurasone (NAPP Chemicals, Lodi, NJ). Liver samples were subsequently analyzed for total fat (FAT) and triacylglycerol (TAG) content, and also to quantify the abundance of messenger ribonucleic acid (mRNA) of genes in liver encoding enzymes *pyruvate carboxylase* (PC), *phosphoenolpyruvate carboxykinase* (PEPCK) and *microsomal triacylglycerol transfer protein* (MTP).

Radioimmunoassays

Second antibody preparation. Florida native sheep managed at the DRU were used to develop specific second antibodies for use in specific radioimmunoassays (RIA) of hormones and IGF-I. Rabbit gamma globulin (Sigma Chemical Co., St. Louis, MO. # R-9133) and guinea pig gamma globulin (Sigma Chemical Co., St. Louis, MO. # R-9135) were injected into sheep to induce production of second antibodies. Guinea pig gamma globulin (20-25 mg) or rabbit gamma globulin (30-40 mg) were added to 5 to 15 mL distilled water in an Erlenmyer flask and stirred on a magnetic stir plate until solubilized. Freund's complete adjuvant (Sigma Chemical Co., St. Louis, MO) was added in equal amounts to each solution and mixed using an OMNI 2000 micro-blender (OMNI International, Waterbury, CT) for approximately 1 min. About 5 ml of this homogenized mixture were injected subcutaneously (SC) over the left and right shoulders of each sheep in three to four separate locations. Ten to 14 d after initial injection the procedure was repeated, except Freund's incomplete adjuvant was mixed with the gamma globulins prepared as described above. After 14 d the sheep were bled by jugular venipuncture; approximately 400 ml of blood were taken using an 18 gauge butterfly needle and 60 cc syringe to withdraw the blood. Blood was dispensed into 40 ml screw top centrifuge tubes, placed on ice and left overnight to clot (16 h at 4 °C). Tubes then were centrifuged at 3000 RPM for 30 min (RC-3B refrigerated centrifuge, H 600A rotor, Sorvall Instruments, Wilmington, DE). Serum was pooled and frozen. Sheep were bled again after an additional 28 d and thereafter at irregular intervals. Sheep were re-injected with gamma globulins in Freund's incomplete adjuvant (Sigma Chemical Co., St. Louis MO) at about 3 to 6 mo intervals. This procedure provided large batches of sheep anti-rabbit gamma globulin serum (SAR) or sheep anti-guinea pig gamma globulin serum (SAGP) for use as the second antibody in the specific RIA procedures.

Insulin, somatotropin and IGF-I iodination and protein separation. The iodination procedure used was similar for all hormones. For insulin, bovine insulin (100-300 µg # 118F-4826; Sigma Immunochemicals, St Louis, MO) was weighed and mixed with an equal quantity of 5 mM HCl (pH=2.5) to yield solutions that contained 1 µg/µL. For somatotropin (ST), bovine ST (100-300 µg; USDA Reproduction Lab, # AFP 5200) was weighed and mixed with an equal quantity of 0.01 M $NaHCO_3$ (pH=9.5) to yield solutions that contained 1 µg/µL. Then, an equal amount of 0.01 M phosphate buffer (2 mL of 0.5 M phosphate buffer [23.04 g sodium phosphate monobasic, 70.98 g sodium phosphate dibasic and 1.0 g sodium azide in 1 L distilled H_2O, pH=7.5] in 100 mL distilled H_2O, pH=7.5) was added to give a final concentration of 0.5 µg insulin or 0.5 µg ST/µL solution, respectively. For insulin-like growth factor-I (IGF-I), bovine IGF-I (25 µg Cat# 01-189; Upstate Biotechnology, Lake Placid, NY.) was dissolved in 250 µL of 0.1 M acetic acid to give stock 0 (0.1 µg/µL). Then, following procedures outlined, 10 µL of each solution containing insulin, ST or IGF-I were frozen in microcentrifuge vials (Fisher Scientific, 1.0 mL, flat top) for use in the iodination procedure.

To separate iodinated insulin (INS^{I125}) and IGF-I (IGF-I^{I125}) from free iodine, a 16 mL (20 cm long) chromatography column (Kontes Glass Company, Vineland, NJ, # 420400-1020) was used. To separate iodinated ST (ST^{I125}) from free iodine, a 40 mL (50 cm) chromatography column (Kontes Glass Company, Vineland, NJ, # 420401-1050) was used. For insulin and IGF-I, and for ST, Sephadex G-50 and G-75 (Sigma Chemical Co., St. Louis MO) dispersed in distilled H_2O was added to the column until the Sephadex packing was about 2.5 cm of the top of the column.

Subsequently, the insulin column was washed with 5 mL of borate/BSA buffer (16.50 g boric acid, 5.40 g NaOH, 6.0 mL 12 N HCl, 200 mg Merthiolate and 0.5% BSA in 2 L distilled H_2O, pH=8.0); ST column was washed with 5 mL of 0.1 M phosphate/BSA buffer (9.216 g sodium phosphate monobasic, 28.392 g sodium phosphate dibasic, 2 g sodium azide, 16.8 g NaCl and 5 g BSA in 2 L distilled H_2O, pH=7.5); and IGF-I column was washed with 5 mL of phosphate/BSA buffer (200 mg protamine, 4.14 g sodium phosphate, 10 mL of a 2% solution of sodium azide, 3.72 g EDTA and 2.5g BSA in 1 L distilled H_2O, pH=7.5). Following this first wash, columns for each hormone were re-washed (5 mL) with 0.01 M phosphate buffer. Phosphate buffer was retained at the top of the column until it was used for protein separation.

Immediately prior to the iodination, 3 mg of chloramine-T and 5 mg sodium metabisulfite were weighed into 12x75 mm borosilicate glass tubes (Fisher Scientific, Pittsburgh, PA) then each chemical was dissolved individually in 1 mL 0.5 M phosphate buffer. Sequentially, radioactive iodine (I^{125} 10 μL, 1 mci/10 μL), 0.5 M phosphate buffer (20 μL), and 10 μL chloramine-T were added to the vial that contained the insulin, ST or IGF-I. The iodination reaction was allowed to proceed for 15-20 sec, during which time contents were mixed by finger tapping; then 10 μL sodium metabisulfite were added to allow complete oxidation of chloramine-T. The final reaction mixture then was transferred to the top of the Sephadex column. Following the transfer, the reaction vial was rinsed with 50 μL 0.01 M phosphate buffer (for all hormones or growth factor) to ensure complete transfer of the iodinated proteins.

To collect iodinated insulin, borosilicate tubes (13x100 mm) numbered 1 to 35 were filled with 500 μL of borate/BSA buffer; for ST, tubes were numbered 1 to 50 and 500 μL of 0.1 M phosphate/BSA buffer were added; and for IGF-I, tubes were numbered 1 to 35 and 500 μL of phosphate/BSA buffer were added. Tubes were placed in a fraction collector to receive 20 drops from the Sephadex column. Aliquots (10 μL) of each eluted fraction were transferred to a second set of tubes to identify elution peaks by counting radioactivity using a Tracor Analytic Gamma Counter (Nuclear-Chicago, Gamma Trac 1191, G. D. Searle and Co., Des Plaines, IL). Tubes that contained the first large peak, which corresponded to I^{125} bound to protein, were pooled. The second large peak was free I^{125}. The I^{125}-INS, -ST and -IGF-I were stored at 4 °C and used within 15 d of iodination.

Insulin Assay

A double antibody radioimmunoassay procedure, as described by Soeldner and Sloane (1965), modified and validated by Malven et al. (1987), was used for assay of insulin in plasma samples. Purified insulin (Sigma Immunochemicals, St. Louis, MO. R#118F-4826) was weighed (~100 μg) as stock insulin and dissolved in 5 mM HCL (pH=2.5). The stock INS (10 μg/10 μL) was diluted in borate/BSA buffer to give a final concentration of 100 ng/ml for preparation of standards that contained 0.1, 0.2, 0.3, 0.5, 0.8, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 and 15.0 ng INS/mL. The first antibody (primary), guinea pig anti-bovine insulin, was obtained from Sigma Chemical Co. (St. Louis, MO). It was dissolved in borate/BSA buffer (1:20,000). The second antibody, sheep anti-guinea pig (SAGP) gammaglobulin, was prepared as described, and diluted 1:2 to 1:5, as appropriate, for complete precipitation of the RIA reaction complex, in borate/EDTA buffer (16.50 g Boric acid, 5.40 g NaOH, 6.0 mL 12 N HCl, 200 mg Merthiolate and 1.86 g EDTA disodium salt diluted in 100 mL of borate buffer, pH=8.0). Normal guinea pig serum (NGPS) was diluted 1:200 in borate/BSA buffer for use in the assay.

Aliquots of insulin standards (100 µL) and plasma samples (125 µL) were diluted with 200 and 175 µL borate/BSA buffer, and assayed in triplicate and duplicate, respectively, in 12x75 mm borosilicate tubes. Immediately after samples were pipetted, 100 µL diluted primary antiserum were added to all tubes, except for total count, NSB-B and NSB-P tubes. Eight to 12 h later 100 µL iodinated insulin ($INS^{I125} \cong 30{,}000$ CPM) were added to all tubes. Tube arrangements and the volumes used in all individual assay tubes are in Table 2.

Table 2. Arrangement of tubes for radioimmunoassay of insulin.

Tubes[1]	Buffer	Sample	1st Ab	I^{125}	Plasma
TCT	---	---	---	100	---
NSB-B	400	---	---	100	---
NSB-P	275	---	---	100	125
Zero	300	---	100	100	---
Standard	200	100	100	100	---
Sample	175	125	100	100	---

[1] All volumes in µL. TCT is the total count tube. NSB-B is the buffer nonspecific binding tube. NSB-P is the plasma nonspecific binding tube. Zero is reference tube, no insulin added.

After pipetting, all assay tubes were incubated for a total of 24 h at 4 °C. Following incubation period, 100 µL each of SAGP and NGPS were added to all tubes, except the total count tubes, and then incubated for additional 10 min. Then, 750 µL of 15% polyethylene glycol (PEG, Carbowax PEG 8000, Fisher Scientific, Pittsburgh, PA) in borate buffer (16.50 g boric acid, 5.40 g NaOH, 6.0 mL 12 N HCl and 200 mg Merthiolate in 2 L distilled H_2O, pH=8.0) were added to all tubes except the total count tubes, and vortexed. Following 5 to 10 min incubation the tubes were centrifuged at 3000 RPM for 30 min at 4 °C (RC-3B refrigerated centrifuge, H 600A rotor, Sorvall Instruments, Wilmington, DE), decanted, and inverted on absorbent paper to drain. After tubes had dried at room temperature, bound radioactivity in tubes was measured using a Packard® auto gamma counter (model B-5005). Results were calculated using the spline curve fit for radioimmunoassay data processing procedure with 6.67 as a correction multiplier to correct concentrations to ng/ml. The intra- and inter-assay variations for insulin radioimmunoassay were 5.78 and 3.6%, respectively.

Somatotropin Assay

A double antibody radioimmunoassay procedure, as described by Garcia-Gavidia (1998), was used for assay of ST in plasma samples. Purified bovine ST, supplied by USDA (AFP-5200), was weighed (1 mg) as stock ST and diluted in 10 mL of 0.01 M $NaHCO_3$ (pH=9.5) to give a concentration of 100,000 ng/mL. Two (2) mL of the stock ST were was diluted with 18 mL of 0.1M phosphate/BSA buffer to give a concentration of 10,000 ng/mL. Then, 1 mL of this solution was diluted with 99 mL of 0.1 M phosphate/BSA buffer to give a solution with final concentration of 100 ng/mL for preparation of standards. A set of 12 standards was prepared to contain 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, and 5.0 ng ST/100 µL. For first antibody preparation, Rabbit anti-ovine ST (National Hormone and Pituitary Program) was diluted in 0.1 M phosphate/BSA buffer (1:40,000). Second antibody (sheep anti-rabbit serum [SAR]) was diluted 1:4 in phosphate/EDTA buffer without BSA. Normal rabbit serum (NRS) was diluted 1:100 in 0.1 M phosphate/BSA buffer for use.

Aliquots of ST standards and plasma samples (100 µL) were diluted with 200 µL 0.1 M phosphate/BSA buffer, and assayed in triplicate and duplicate in 12x75 mm borosilicate tubes, respectively. Immediately after samples were pipetted, 100 µL of first antibody and 100 µL of iodinated ($ST^{I125} \cong 25,000$ cpm) were pipetted into all tubes, except for total count, NSB-B and NSB-P, tubes that received no first antibody. After incubation for 24 h at 4°C, 100 µL each of SAR and NRS were added to all tubes, except the total count tubes, and mixed (Table 3). Then, 1.0 mL of 6.0% polyethylene glycol (PEG, Carbowax PEG 8000, Fisher Scientific, Pittsburgh, PA) in 0.1 M phosphate buffer (without BSA) was added to all tubes, except total count, and vortexed for 1 min. Following a 5 to 10 min incubation the tubes were centrifuged at 3000 RPM for 30 min at 4 °C (RC-3B refrigerated centrifuge, H 600A rotor, Sorvall Instruments, Wilmington, DE), decanted, and inverted on absorbent paper to drain.

After tubes had dried at room temperature, bound radioactivity in tubes was measured using a Packard® auto gamma counter (model B-5005). Results were calculated using the spline curve fit for radioimmunoassay data processing procedure with 10 as a correction multiplier to correct concentrations to ng/ml. The intra- and inter-assay variations for somatotropin insulin radioimmunoassay were 5.69 and 3.55%, respectively.

Table 3. Arrangement of tubes for radioimmunoassay of ST.

Tubes[1]	Buffer	Sample	1st Ab	I^{125}	Plasma
TCT	---	---	---	100	---
NSB-B	400	---	---	100	---
NSB-P	300	---	---	100	100
Zero	300	---	100	100	---
Standard	200	100	100	100	---
Sample	200	100	100	100	---

[1] All volumes in µL. TCT is the total count tube. NSB-B is the buffer nonspecific binding tube. NSB-P is the plasma nonspecific binding tube. Zero is reference tube, no ST added.

IGF-I Assay

A double antibody radioimmunoassay, as described by Abribat et al. (1990) and modified for sample extraction by method of Daughaday et al. (1980) and Enright et al. (1990), was used for determination of IGF-I in plasma samples.

Extraction of IGF-I from binding proteins. The method of Enright et al. (1990) was used for the extraction of IGF-I from its binding proteins in plasma samples. An extraction mixture of ethanol, acetone and acetic acid (EAA 60:30:10 by volume) was used for extraction. Exactly 100 µL plasma and 100 µL pool plasma for NSB-P were pipetted into 12x75 mm borosilicate tubes, then 400 µL of extraction mixture were pipetted into these tubes. The mixture was vortexed for 15 sec and then allowed to stand for 30 min at room temperature. After this wait, tubes were centrifuged at 3000 RPM for 30 min at 4 °C (RC-3B refrigerated centrifuge, H 600A rotor, Sorvall Instruments, Wilmington, DE). Then, 125 µL of the supernatant were transferred into 12x75 mm polystyrene tubes (Sarstedt Inc., Newtown, NJ), and 50 µL of 0.855 M TRIZMA base and 175 µL of the phosphate/BSA buffer were added to each tube to make the final plasma dilution of 1:14.

Assay. Highly purified recombinant bovine IGF-I (25µg), obtained from Upstate Biotechnology (Lake Placid, NY, Cat# 01-189) was dissolved in 250 µL of 0.1 M acetic acid to give stock 0 (100 ng/µL), then aliquoted into microcentrifuge tubes (1µg/10 µL) and frozen. To make stock 1, 10 µL of stock 0 were added to 490 µL of phosphate/BSA buffer to give 2 ng/µL. Stock 2 was prepared by adding 10 µL of stock 1 to 990 µL of phosphate/BSA buffer to give a final concentration of 20 pg IGF-I/µL. Standards were prepared from stock 2 to contain 50, 100, 200, 400, 600, 800, 1000, 1500, 2000, 3000, 4000 and 5000 pg IGF-I/mL. The first antibody, rabbit anti-bovine IGF-I (Lot# AFP4892898) was diluted 1:160,000 in phosphate/BSA buffer; second antibody (sheep anti-rabbit serum [SAR]) was diluted to 1:3 in phosphate/BSA buffer; and normal rabbit serum (NRS) was diluted 1:50 in phosphate/BSA buffer.

Aliquots of plasma extract (10 µL) and standards (100 µL) were mixed with 190 and 100 µL, respectively, of phosphate/BSA buffer in 12x75 polystyrene tubes, and assayed in duplicate and triplicate. Immediately after pipetting, 100 µL of first antibody were added to all tubes, except total count, and NSB-B and NSB-P tubes. Following the addition of first antibody, 100 µL iodinated IGF-I ($\text{IGF-I}^{I125} \cong 20{,}000$ cpm) were added to all tubes. After a 24 h incubation at 4 °C incubation, 50 µL each of SAR and NRS were added to all tubes, except the total count tubes (Table 4), mixed and allowed to stand 30 min, and then 1 mL of 6% polyethylene glycol in phosphate/BSA buffer (PEG, Carbowax PEG 8000, Fisher Scientific, Pittsburgh, PA) was added. Tubes were vortexed and allowed to stand for an additional 15 min. Following incubation, tubes were centrifuged at 3000 RPM for 30 min at 4 °C (RC-3B refrigerated centrifuge, H 600A rotor, Sorvall Instruments, Wilmington, DE), decanted, and inverted on absorbent paper to drain.

Table 4. Arrangement of tubes for radioimmunoassay of IGF-I.

Tubes[1]	Buffer	Sample	1st Ab	I^{125}	Plasma Extract
TCT	---	---	---	100	---
NSB-B	300	---	---	100	---
NSB-P	290	---	---	100	10
Zero	200	---	100	100	---
Standard	100	100	100	100	---
Sample	190	10	100	100	---

[1] All volumes in µL. TCT is the total count tube. NSB-B is the buffer nonspecific binding tube. NSB-P is the plasma extract nonspecific binding tube. Zero is reference tube, no IGF-I added.

After tubes had dried at room temperature, bound radioactivity in tubes was measured using a Packard® auto gamma counter (model B-5005). Results were calculated using the spline curve fit for radioimmunoassay data processing procedure with 1.4 as a correction multiplier to correct concentrations to ng/ml of plasma. The intra- and inter-assay variations for somatotropin insulin radioimmunoassay were 7.08 and 3.6%, respectively.

Calcium Assay

The method of Miles et al. (2001) for mineral analysis by flame atomic absorption spectrophotometry was used to determine concentration of calcium in plasma samples. Calcium reference standard solution (1000 ppm, Fisher Scientific, Pittsburgh, PA, # SC191-500) was diluted 10-fold in a volumetric flask to prepare 100 ppm Ca stock solution. From the stock Ca, standards containing 5, 10 and 15 ppm were prepared (Table 5).

To prepare 5% Lanthanum (La), 117.3 g Lanthanum Oxide (La_2O_3) were added to a 2 liter pyrex beaker and dissolved in 500 mL of concentrated HCl in a fume hood. After the solution released O_2 and Cl_2 and all La was in solution, it was transferred to a 2 L volumetric flask and brought to 2 L with distilled water to prepare the 5% La solution.

Table 5. Standards for determination of calcium by flame atomic absorption spectometry.

	Standard, ppm			
Ingredient	*0*	*5*	*10*	*15*
100 ppm Ca stock, mL	0	5	10	15
50% TCA, mL	18	18	18	18
5% La, mL	18	18	18	18

TCA=Trichloracetic acid, La=Lanthanum

To prepare a 50% TCA solution, 500 g of trichloracetic acid (TCA) was dissolved in distilled water in a 1 L volumetric flask. Then, 200 mL 5% La and 200 mL 50% TCA were transferred to a 1 L volumetric flask and distilled water added to give 1 liter of a 1% La-10% TCA solution.

The assay consisted of pipetting 500 μL of plasma samples into polypropylene tubes (Sarstedt Inc., Newton, NJ). To precipitate proteins, 4.5 mL of the 1% La-10% TCA solution were added to each tube containing sample, then they were vortexed for 20 sec and finally all tubes centrifuged at 3000 RPM for 20 min at 4 °C (RC-3B refrigerated centrifuge, H 600A rotor, Sorvall Instruments, Wilmington, DE). About 4 mL of filtrate were transferred into polypropylene tubes and samples were analyzed for concentrations of Ca using a Flame Atomic Absorption Spectrophotometer (Perkin-Elmer Model 5000).

Glucose Assay

The method of Raabo and Terkildsen (1960) was used for assay of glucose in the plasma samples. Concentrations of glucose were determined using a Sigma Diagnostics® Glucose Enzymatic kit (# 510) purchased from Sigma Chemical Co. (St. Louis, MO). Specific concentrations of standards used were: 0, 25, 50, 75 and 100 mg of glucose/dL. These were prepared by diluting the glucose standard solution provided (100 mg/dL) with distilled water to achieve the desired standard concentrations at the same time plasma samples or standards were deproteinized. Standard dilutions used in the assay are in Table 6.

Table 6. Standard dilutions used in the glucose assay.

Concentration of Standards (mg/dL)	GLC Standard Solution (μL)	Amount of distilled water (μL)
0	0	1000
25	25	975
50	50	950
75	75	925
100	100	900

After preparing the solutions, 100 μL of standards and plasma were added to 12x75 mm borosilicate tubes containing 900 μL of distilled H_2O. The precipitation of proteins was effected by adding 500 μL of barium hydroxide solution (0.3N) and 500 μL of zinc sulfate solution (5%) to all tubes, including standards. The tubes were vortexed then centrifuged at 3000 RPM for 15 min at 4 °C (RC-3B refrigerated centrifuge, H 600A rotor, Sorvall Instruments, Wilmington, DE).

Aliquots of the 1:20 protein-free plasma filtrate (20 μL of standards and samples) were added to wells in a 96-well (0.45 ml well capacity) polypropylene plate (Fisher

Scientific, Pittsburgh, PA) in triplicate and duplicate, respectively. Then 200 µL of combined enzyme-color reagent solution [(1 capsule of PGO enzymes + 1.6 ml o-dianisidine dihydrochloride - color reagent solution) + 100 ml of distilled water] were added to each well. The plasma protein-free filtrate and combined reactants were incubated in a constant temperature oven (Model DN-41, American Scientific Products, Japan) for 30 min at 37°C. After incubation, the absorbance was read in an Automated Microplate Reader (model EL 309, Bio-Tek Instruments, INC., Laboratory division, Winooski, VT) using blank as reference at 450 nm wavelength. Arrangement of tubes for this assay is described in Table 7. Linear regression of absorbance and glucose concentration was used to determine the concentration (glucose/dL) in plasma samples; final concentrations were expressed as mg/dL.

Table 7. Arrangement of tubes and solutions used to make plasma protein-free filtrates.

Tubes	Distilled Water (µL)	Sample (µL)	Barium Hydroxide solution (µL)[1]	Zinc Sulfate solution (µL)[2]	TOTAL (mL)
Standard	900	100	500	500	2
Sample	900	100	500	500	2

[1] Barium hydroxide, 0.3 N; [2] Zinc Sulfate, 5% solution.

Nonesterified Fatty Acid (NEFA) Assay

An *in vitro* enzymatic method described by Johnson and Peters (1993) was used for assay of NEFA in all plasma samples. Concentrations of NEFA were determined using a WAKO® NEFA C Test kit (# 994-75409E) purchased from Wako Chemicals INC., Richmond, VA. Color reagent solution A, provided in the kit, was prepared by combining 10 mL of diluted color reagent A with 13.3 mL 50 mM phosphate buffer (4.6g sodium phosphate monobasic, 14.2 g sodium phosphate dibasic in 500 mL distilled water, pH ~6.9). Color reagent solution B was prepared by combining 20 mL of diluted color reagent B provided in the kit with 33.3 mL of 50 mM phosphate buffer. Vials containing reagents A and B were mixed gently and stored at 5 °C for up to 2 wk.

Specific concentrations of standards (0, 200, 400, 600, 800 and 1000 µEq NEFA/L) were prepared by diluting the NEFA standard solution provided (1000 µEq/L) with 0.9% saline solution (9 g of NaCl in 1000 mL distilled water) to achieve the desired standard concentration (Table 8).

Table 8. Standard dilutions for NEFA determination.

Concentration of Standards (µEq/L)	Oleic Stock Solution (1.0 mM, µL)	0.9% Saline (µL)
0	0	500
200	100	400
400	200	300
600	300	200
800	400	100
1000	500	0

For the assay, 96 well (0.45 ml well capacity) polypropylene plates (Fisher Scientific, Pittsburgh, PA) were used. Aliquots (3.0 µL of standards and samples) were pipetted in triplicate and duplicate, respectively. Following pipetting, 60 µL of Wako Reagent solution A were added to each well and plates were incubated for 30 min at 25 °C in a constant temperature oven (Model DN-41, American Scientific Products, Japan). After incubation, 120 µL of Wako Reagent solution B were added to each well, followed by an additional incubation period of 30 min at 25 °C. After this step, the microplate was allowed to stand for 5 min at room temperature then absorbance for each well was read in an Automated Microplate Reader (Model EL 309, Bio-Tek Instruments, INC., Laboratory

Division, Winooski, VT), using blank as reference at 550 nm wavelength. Linear regression of absorbance and NEFA concentration was used to determine the concentration (NEFA/L) in plasma samples with final concentrations expressed as µEq/L.

β-Hydroxybutyrate (B-HBA) Assay

An *in vitro* enzymatic method, as described, by Hansen and Freier (1978) was used for assay of B-HBA in plasma samples. Concentrations of B-HBA were determined using a Pointe Scientific® β-Hydroxybutyrate Kit (# H7587-01; H7587-02) purchased from Pointe Scientific, Inc., Lincoln Park, MI. Specific standard concentrations used (2.08, 10.41 and 41.66 mg/dL) were provided in the kit. Preparation of the working reagent consisted of adding 10 parts of R1 with 1.5 parts of R2, both were provided in the kit.

Aliquots of 3 µL of standard and samples were added to wells in a 96-well (0.45 ml well capacity) polypropylene plate (Fisher Scientific, Pittsburgh, PA) in triplicate and duplicate, respectively. Following pipetting, 120 µL of working reagent solution were added to all wells and plates were incubated for 5 min at 37 °C in a constant temperature oven (Model DN-41, American Scientific Products, Japan). After incubation, the absorbance was read in an Automated Microplate Reader (Model EL 309, Bio-Tek Instruments, INC., Laboratory division, Winooski, VT) using blank as reference at 490 nm wavelength. Linear regression of absorbance and B-HBA concentration was used to determine the concentration (B-HBA/dL) in plasma samples with final concentrations expressed as mg/dL.

Total Lipid Determination of Liver Samples

Percentage of total lipid in liver was determined by a method described by Drackley et al. (1991). Two days before weighing the liver tissue, two screw-cap tubes (15x150 mm Pyrex Brand Culture Tubes with threaded end, Fisher Scientific, Pittsburg, PA) were dried overnight at 105 °C in a constant temperature oven (Model DN-41, American Scientific Products, Japan). The dried tubes then were weighed twice and weights recorded. Subsequently, ≅100 mg of freshly thawed liver was weighed and added to each of two screw-cap tubes (10 mL, 13x100 mm Fisherbrand Borosilicate Glass Tubes with threaded end, Fisher Scientific, Pittsburgh, PA). To each tube, 10 mL of a chloroform and methanol (2:1, vol/vol) mixture were added and tubes were vortexed. After this step, liver tissues were homogenized using a PowerGen tissue homogenizer (PowerGen 700, Fisher Scientific, Pittsburgh, PA) for 30 sec, and then the tubes were placed in a horizontal shaker (GCA/Precision Scientific, Chicago, IL) at room temperature for 5 min.

To each tube, 4 mL of distilled H_2O were added, and the tubes were vortexed and then centrifuged at 1700 RPM for 5 min at 4 °C (RC-3B refrigerated centrifuge, H 600A rotor, Sorvall Instruments, Wilmington, DE). After centrifugation, the top layer (water-methanol) was aspirated and discarded, and the bottom layer (chloroform-lipids) was poured through a Buchner funnel containing a Whatman 425 mm diameter filter paper (Cat. # 1822042) and collected in pre-weighed 15x150 mm screw-cap tubes. Extraction tubes were rinsed with an additional 9 mL of chloroform and this was poured though the Buchner funnel and collected in the tubes. Following filtration, tubes containing the chloroform-lipid mixture were dried under a stream of nitrogen in a water bath at 40-50

°C (Circulating Water Bath, GCA/Precision Scientific Group, Chicago, IL) for 30-40 min, and then dried at 55 °C for 5 min in a constant temperature oven (Model DN-41, American Scientific Products, Japan). Dried tubes were re-weighed to determine total dry lipid weight. Lipid content (mg) was expressed on a wet weight basis relative to weight of liver originally extracted.

Triacylglycerol (TAG) Determination of Liver Samples

The lipid extracted from liver samples by the procedure described above was redissolved in 2 mL of a 3:2 (vol/vol) hexane:isopropanol mixture, and concentrations of TAG were measured colorimetrically as described by Foster and Dunn (1973). Specific concentrations of standards used were 0, 0.02, 0.04, 0.08, 0.16 mg of TAG/dL. These standards were prepared by diluting the TAG standard solution (200 mg/dL, Pointe Scientific, Inc., Lincoln Park, MI) with isopropanol and distilled H_2O. Standard dilutions used in the assay are in Table 9.

For assay, 60 µL aliquots of sample were pipetted, in duplicate, into 15x75 mm borosilicate tubes that contained 1940 µL of isopropanol, 80 µL of distilled H_2O and ≅160 mg of TAG purifier (Alumina, Sigma-Aldrich, St. Louis, MO) that had been extracted with methanol. All tubes were vortexed for 30 sec and allowed to stand until the TAG purifier settled out, and then all tubes were centrifuged at 1000 RPM for 5 min at 4 °C (RC-3B refrigerated centrifuge, H600A rotor, Sorvall Instruments, Wilmington, DE). Following this initial step, all samples were saponified by transferring 1 mL of supernatant mixture into 12x75 mm borosilicate tubes. Then, 250 µL of 1 N potassium hydroxide solution were added to all tubes (just above the liquid level), followed by an incubation period of 5 min at 60 °C in a constant temperature oven (Model DN-41, American Scientific Products, Japan). After cooling the sample to room temperature in tap water, 250 µL of periodate solution (125 mg sodium m-periodate diluted in 50 mL of 2 N acetic acid solution) were added to all tubes, and these tubes were allowed to incubate at room temperature for 10 min. Subsequently, 1.5 mL of color reagent solution (20 mL ammonium acetate, 40 mL isopropanol and 150 µL of acetylacetone) were added to all tubes, the solution in tubes then was mixed and incubated for 30 min at 60 °C in a constant temperature oven (Model DN-41, American Scientific Products, Japan).

Aliquots of 200 µL of corresponding standard and samples were added to wells in a 96-well (0.45 ml well capacity) polypropylene plate (Fisher Scientific, Pittsburgh, PA) in triplicate and duplicate, respectively. After pipetting, the absorbance was read in an Automated Microplate Reader (Model EL 309, Bio-Tek Instruments, INC., Laboratory division, Winooski, VT) using blank as reference at 405 nm wavelength. Linear regression of absorbance and TAG concentration was used to determine the concentration (mg TAG/dL) of total lipid extracted from the tissue. To obtain the total concentration of TAG in the reconstituted sample, all results were multiplied by 35, which was the

Table 9. Standard dilutions used for determination of TAG.

Standard Concentration (mg/dL)*	Standard (µL)	Isopropanol (µL)	Distilled H_2O (µL)
0	0	2000	80
0.02	10	1990	80
0.04	20	1980	80
0.08	40	1960	80
0.16	80	1920	80

* Pointe Scientific, Inc., Lincoln Park, MI

correction factor. Final concentrations (mg) were expressed on a wet basis relative to total liver weight originally extracted (mg).

Messenger Ribonucleic Acid (mRNA) Determination by DOT BLOT Procedure

The method described by Selberg (2002) was used to quantify the abundance of messenger ribonucleic acid (mRNA) of genes in liver encoding the enzymes *pyruvate carboxylase* (PC), *phosphoenolpyruvate carboxykinase* (PEPCK) and *microsomal triacylglycerol transfer protein* (MTP).

Extraction of mRNA. Total liver cellular RNA from 6, 6, 7 and 6 cows randomly selected from TRT I, II, III and IV was isolated using TRIzol reagent (Invitrogen Life Technologies, Grand Island, NY). The procedure was initiated by pipetting 3 mL of TRIzol into sterile 15 mL conical tubes (Fisher Scientific, Pittsburgh, PA). Following pipetting, 250-300 mg of partially thawed liver samples were added to appropriate tubes, homogenized by 3-5 sec bursts using a tissue homogenizer (PowerGen 700, Fisher Scientific, Pittsburgh, PA), and three equal aliquots of 1 mL from each sample were transferred into sterilized 1.5 mL polypropylene microcentrifuge tubes (Fisher Scientific, Pittsburgh, PA). After a 5 min incubation at room temperature, 200 μL of chloroform were added to each tube, which then were vigorously shaken and allowed to incubate for an additional 3 min at room temperature. Subsequently, all tubes were centrifuged at 13,000 x g for 15 min at 4 °C (Biofuge Fresco, Heraeus Instruments, Ashville, NC) After centrifugation, the colorless upper aqueous phase of the mixture (≅ 600 μL) was carefully transferred to a fresh 1.5 mL microcentrifuge tube, 500 μl of isopropanol were added, capped and mixed gently by inversion (5 inversions). Tubes then were incubated at room temperature for an additional 10 min, and then centrifugated at 13,000 x g for 10 min at 4

°C (Biofuge Fresco, Heraeus Instruments, Asheville, NC). The supernatant was carefully decanted by inverting the tubes after centrifugation.

The RNA pellet remaining in the tube was washed with 1 mL of 75% ethanol, mixed by vortexing and centrifuged for an additional 3 min at 12,000 x g at 4 °C (Biofuge Fresco, Heraeus Instruments, Asheville, NC). Following this last step, the supernatant was carefully decanted by inverting the tubes, and the RNA pellet in the tube was allowed to dry for approximately 5 min at room temperature. The RNA pellet was resuspended in 25 μL of pure water (Gibco Ultra Pure® Distilled Water, Grand Island, NY), and the RNA volumes of common samples were combined. To quantify the amount of RNA, a 5 μL aliquot of RNA sample was diluted with 995 μL of the pure water in a 12x75 mm borosilicate tube (Fisher Scientific, Pittsburgh, PA), and absorbance was read in a biophotometer (Brinkmann-Eppendorf® Instruments, Westbury, NY) at 260 nm and 280 nm. The 260 absorbance was multiplied by a correction factor of 6.6 to give final concentrations in μg RNA/μL.

Dot Blot Membrane Transfer. Ten μg (10 μg) of each extracted RNA, were transferred to a corresponding 1.5 mL sterile microcentrifuge tube (Fisher Scientific, Pittsburgh, PA). Following addition of samples, a piece of 0.2 μm nylon membrane (ICN Biomedicals, Inc., Costa Mesa, CA) was briefly wetted in deionized H_2O and soaked in 10X Saline Sodium Citrate (SSC, 1:2 dilution of 20X SSC [175.3 g NaCl, 88.2 g Na-Citrate, 800 mL di-deionized H_2O, pH=7.0 brought to 1 L solution with di-deionized (twice deionized) H_2O] and di-deionized H_2O) for 10 min at room temperature. Following this step, the piece of absorbent paper (previously wetted with 10X SSC) was placed on the top of the vacuum unit of the dot blot apparatus (Minifold I Microsample

Filtration Manifold, Schleicher & Schuell, Keene, NH), and the nylon membrane was placed in the bottom of the sample wells (96) of the manifold apparatus. The two parts of the manifold were clamped together and each well was filled with 500 µL of 10X SSC. The vacuum unit was connected to the vacuum line and a gentle suction was applied to the unit so that all fluid could pass through the nylon filter within 5 min.

Following this procedure, the RNA previously pipetted was diluted with 250 µL of sample denaturation buffer (200 µL 10 mM tris, 5 mL 50% fomamide, 6% formaldehyde and 3.18 mL of di-deionized H_2O), incubated for 5 min at 65 °C in a circulating water bath (Fisher Isotemp Model 210, Fisher Scientific, Pittsburgh, PA) and cooled on ice. After cooling, 250 µL of 20X SSC were added to each sample, mixed thoroughly and gently loaded into wells of the manifold apparatus. Gentle suction was applied so that all samples would pass through the nylon membrane, which would retain the RNA. Each well of the apparatus was rinsed again with 500 µL of 20X SSC, and then the vacuum was turned off.

The nylon membrane was removed from the manifold, wrapped in plastic, and exposed to UV light (Hoefer Macro Vue UV-25, San Francisco, CA) for 90 sec to cross-link the mRNA to the filter. Then, the membrane was removed from the wrap, marked for orientation, placed in a folded absorbent paper and incubated for 1 h at 80°C in a constant temperature oven (Model DN-41, American Scientific Products, Japan). After incubation, the membrane was placed in a plastic bag and stored at 4 °C until hybridization.

Hybridization. The process began with pre-hybridization of the nylon membrane with an ultrasensitive hybridization buffer (ULTRAhyb™, Ambion, Houston, TX) for 2 h at 42 °C in a hybridization tube placed in a hybridization oven (FisherBiotech Hybridization Incubator, Fisher Scientific, Pittsburgh, PA). At 1 h after the procedure began, 1 µL of cDNA probes for the specific enzyme (PC, PEPCK or MTP; gift of Dr. Lokenga Badinga, Department of Animal Sciences, University of Florida) was added to a 500 µL sterile polypropylene microcentrifuge tube that contained 22 µL of di-deionized H_2O. The tube was placed in a dry-bath incubator (Fisher Isotemp, Fisher Scientific, Pittsburgh, PA) at 100 °C for 3 min to boil the contents. Then, the vial was centrifuged for 5 sec at 5,000 x g at room temperature (Biofuge Pico Microcentrifuge, Heraeus Instruments, Asheville, NC) to spot the contents after which it then was placed on ice.

Following this procedure, 15 µL of buffer, 2 µL of dATP, 2 µL of dGTP and 2 µL of dTTP (Random Primers Kit Labeling System, Invitrogen Life Technologies, Carlsbad, CA) and 5 µL of P^{32} were added to the mixture. After addition of the isotope, 1 µL of the Klenow fragment of the Random Primers Kit was added to the mixture, which was mixed by tapping, centrifuged for 5 sec at 5,000 x g at room temperature (Biofuge Pico Microcentrifuge, Heraeus Instruments, Asheville, NC) to spot the contents (≅ 50 µL) and then incubated at room temperature for 1 h.

Subsequently, the contents of the mixture were fractionated using a G-50 sephadex column equilibrated in TE (tris-EDTA buffer, 10 mL 1.0 M tris [pH=8.0], 2 mL 0.5 M EDTA [pH=8.0] in 1 L of di-deionized H_2O, pH=8.0). The column used to separate iodinated probe (PC-, PEPCK- or MTP-P^{32}) from free P^{32} was prepared by placing a glass bead or glass wool plug into the bottom of a glass Pasteur pipette, before adding the Sephadex (Sephadex G-50, Sigma Chemical) that was dispersed in TE. Then the sephadex mixture was added until it reached the top ring area. Subsequently, the probe-P^{32} mixture was added to the top of the column with an additional 200 µL of TE.

Sterile microcentrifuge plastic tubes (1 mL, n=11) were placed in a fraction collector to receive 4 drops each from the Sephadex column after the addition of 100 µL of TE to the column. After fractionation, tubes were checked with a Geiger counter (Model 3 Survey Meter, Ludlum Measurements, Inc., Sweetwater, TX) to identify the peak fraction. The two tubes that contained the first large peak (≥100,000 cpm), which corresponded to P^{32} bound to the probe, were pooled. The pooled fractions were boiled for 3 min at 100 °C in a dry-bath incubator (Fisher Isotemp, Fisher Scientific, Pittsburgh, PA), and 25 µL of combined peak fractions were added to the fluid in the hybridization tube. The probe was allowed to hybridize the membrane overnight.

After hybridization, the membrane was washed at 42 °C in the hybridization oven (FisherBiotech Hybridization Incubator, Fisher Scientific, Pittsburgh, PA) first with 25 mL of 2X SSC/1% SDS (2685 mL of di-deionized H_2O, 300 mL of 20X SSC and 15 ml of 20% SDS) for 20 min, and second with 25 mL of 0.1X SSC/0.1% SDS (2970 mL of di-deionized H_2O, 15 mL of 20X SSC and 15 mL of 20% SDS) for 10 min. After these washes, the membrane was removed, dried on absorbent paper and checked for signal intensity with a Geiger counter (Model 3 Survey Meter, Ludlum Measurements, Inc., Sweetwater, TX). If signal was ≥ 100 cpm, the membrane was allowed to incubate at -80 °C overnight. If the signal was ≤ 100 cpm, the time of incubation was increased to 2-5 d. Equal loading of total RNA for each experimental sample was verified by subsequent rehybridization of filter membrane with ribosomal 18S cDNA probe.

Incubation, Film Development and Membrane Stripping. For incubation, the membrane in a plastic bag, was placed in an X-Ray Cassete with an X-Ray Film (X-OMAT, Eastman Kodak Co., Rochester, NY). After incubation, the film was developed in an X-Ray Film Developer (Konica X-RAY film Processor, Konica Corporation, Tokyo, Japan). Hybridization signals in the film were quantified by densitometry analysis using Adobe Photoshop CS Software (Version 8.0, Adobe Systems Incorporated, Schaumburg, IL), and values obtained (arbitrary units) were used for statistical analysis.

Following film development, the P^{32}-cDNA probe was removed from the membrane by the method of stripping. For that, the membrane was washed twice with 1% SDS solution (10 g SDS in 1 L of di-deionized H_2O) at 100 °C for 15 min (each wash) in a pyrex container placed on a rocker platform (Belco Biotechnology, Vineland, NJ). After stripping, the membrane was ready to be hybridized with another probe.

Calving Variables, Health Status and Reproductive Performance

To evaluate the effects of treatment on calving variables, on health status and on reproductive performance of experimental cows, data from the DRU farm records (calving variables and disease occurrence [health status]) and from Dairy Herd Improvement Association records (DHIA, Dairy Records Management Systems, Raleigh, NC; reproductive activity information) were collected and analyzed retrospectively. In addition, health and reproductive data of an additional 47 non-experimental DRU cows (cohorts) with 2 or more lactations (including the lactation that was initiated during the time period), were included in the analysis, making a total of 150 cow records available to evaluate the effects of treatment on health and reproduction. The number of lactations of cohort cows ranged from 2 to 6, with an average of 3.37.

The calving data obtained from farm records were calf weight (CLFWT) and difficulty of calving (DCV; 1-5 scale: 1-no help needed; 2- slight problem; 3- needed assistance; 4-considerable force; and 5-extreme difficulty, cesarean section needed; DRU management practice scale). Diagnosis of diseases was by skilled farm personnel and also by the Veterinarians of the Large Animals Clinic, College of Veterinary Medicine, University of Florida. With regard to health, the information obtained was disease incidence (new cases) at any point from parturition until 60 DIM. The diseases considered were those of high incidences, and were as defined by Garry (1996), Kelton et al. (1998), Hernadez et al. (2002) and Manske et al. (2002).

A cow was considered to have milk fever (MF) if she displayed all or some of the following clinical signs within 72 h of calving: sternal or lateral recumbency secondary to flaccid paralysis, depression, fine muscle tremors, severe bloat, profound gastrointestinal atony, rapid heart rate and increased rectal temperature (> 39 °C).

A cow was considered to have retained placenta (RP/RFM) when the fetal membranes were visible at the vulva or were identified in the uterus or vagina by vaginal examination more than 12 h after the first observation of the cow following calving.

A cow was considered to have metritis (MET) if she had a postpartum condition characterized by an abnormal uterine discharge (not lochia), vaginal discharge or both, or uterine content (abnormal fluid). A case was considered to be new if the cow did not have an observed case of metritis during the preceding 30 d.

A cow was considered to have primary ketosis (KETO) if she had decreased appetite and if there was evidence of elevated milk, urine or breath ketones in the absence of other concurrent disease. As for metritis, a case was considered to be new if the cow did not have an observed case of ketosis during the preceding 30 d.

A cow was considered to have left or right displaced abomasum (L/R DA) when she had decreased appetite accompanied by an audible, high pitched tympanic resonance (ping) produced by percussion of the left/right abdominal wall between the 9th and 12th ribs.

For mastitis (MAST), a cow was considered to have the disease if she had a visually abnormal milk secretion (i.e., clots, flakes, or watery) from one or more quarters. Abnormal milk in a different quarter or in the same quarter following at least 8 d of normal milk was considered to be a new case.

For lameness (LAME), a cow was considered to be lame if she had signs of arched-back posture and an abnormal gait associated with one of the following foot lesions: interdigital phlegmon (foot root), papillomatous digital dermatitis (foot warts), claw lesions or abnormalities in it growth, heel-horn erosions, sole hemorrhages, white-line hemorrhages and fissures, double soles or sole ulcers.

Finally, a cow was considered to have digestive problems (indigestion, DIG) if she had signs of decreased appetite accompanied by decreased rumen function (motility). Another variable considered was the total number of cows in a experimental group with any disease (SICK) in an experimental group during the first 60 DIM.

With regard to reproductive performance of experimental cows, the data collected during the whole lactation period (305-d) were days to first observed estrus (after parturition), number of inseminations for a confirmed pregnancy and days open. With these data, it was possible to develop the following variables: A- conception rate at first service (insemination; CRFS); B- pregnancy rate (number of cows that were pregnant in a experimental group within 365 d after parturition, PR); C- calving to conception interval (number of days between parturition and the beginning of a confirmed pregnancy; CCI); and D- Services (inseminations) per conception (SPC). All health and reproductive data were complied in a spreadsheet software program (EXCEL®, Microsoft Corp., Seattle, WA) for subsequent statistical analyses.

Statistical Analyses

Productive Responses, Physiological Changes, Calving Information and Reproductive Performance. These data were analyzed by least squares analysis of variance using the GLM and MIXED procedures of SAS® (SAS Institute, 2001) to determine effects of treatment (TRT, periods of bST supplementation: I [control], II [bST Postpartum], III [bST Prepartum], and IV [bST Pre and Postpartum]) and calving season (SEA; I- August, September, October; II- November, December, January, February) on the dependent variables. Significance for mean variables was declared at $P \leq 0.05$ and tendencies also included at $P > 0.05$ and ≤ 0.10. The PROC MIXED analyses was used to estimate individual daily and/or weekly least squares means for specific variables and treatments (Littell et al., 1998). The covariance used in the mixed models to evaluate variations of effects between and within animals was specified by RANDOM and REPEATED statements, respectively. In addition, PROC CORR procedure and the MANOVA statement in the PROC GLM procedure of SAS® (SAS Institute, 2001) were used to estimate gross and residual correlations between variables, respectively.

Because of the design of the experiment, the time periods considered were prepartum transition period (-3 to 0 wk and -21 d to calving), and the postpartum transition period. For the postpartum period, dependent variables were analyzed in four phases. These periods evaluated were 0-28, 0-63, 64-150 and 0-150 DIM. For milk yield (MY), data were analyzed from 0-63, 64-150 and from 0-150 DIM (previous periods combined). In addition, milk production during the entire lactation [actual MY(305-d)] also was analyzed. With regard to statistical analysis of percentages of fat (FAT), protein (PROT), and the somatic cell count (SCC) in milk, they were analyzed for the time period 0-63 DIM.

Body weights (BW) and body condition scores (BCS) were analyzed during the prepartum period, and from 0-63, 64-150 and 0-150 DIM. For plasma concentrations of insulin, somatotropin (ST) and insulin-like growth factor-I (IGF-I), and for glucose concentration in plasma, the data were analyzed during the prepartum period and during 0-63 DIM. For plasma concentrations of calcium (Ca), non-esterified fatty acids (NEFA) and β-hydroxybutyrate (B-HBA), the time periods considered for analysis were prepartum period and during 0-28 DIM.

The liver measures percentage of total fat (LFAT), percentage of triacylglycerol (TAG) and abundance of messenger ribonucleic acid (mRNA; gene expression) of the three liver enzymes *pyruvate carboxylase* (PC), *phosphoenolpyruvate carboxykinase* (PEPCK) and *microsomal triacylglycerol transfer protein-1* (MTP) were combined in a single analysis. The time considered for calving information was around parturition and the variables evaluated were colostrum immunoglobulin content, calf birth weight, and difficulty of calving. For reproductive performance variables calving to conception interval (CCI) and services (inseminations) per conception (SPC), the whole lactation period was considered.

The first mathematical model included bST, season, and the two-way interaction of bST*season effects on the dependent variables MY, FAT, PROT, SCC, BW, BCS, insulin, ST, IGF-I, Ca, glucose, NEFA and B-HBA. Least squares means were obtained using the overall model with the inclusion of the covariate week or day, to the highest order, as appropriate. Sources of variation included in this model were tested by using cow nested in bST*season as the error term. The complete mathematical model used for this analyses were:

$$Y_h = a + B_i + S_j + BS_{ij} + Cow(BS_{ij}) + I_m + E_{ijl}$$

Where:

Y_h: Estimated value of dependent variable
a: Intercept
B_j: Bovine somatotropin (bST) treatment effect
S_j: Calving season
BS_{ij}: bST by season interaction
Cow $(BS_{ij})_k$: Cow nested within bST treatment by season
I_m: Covariate (week or day)
E_{ijl}: Random error

The second model evaluated bST, season and the two-way interaction of bST*season effects on the dependent variables actual MY(305-d), colostrum, calf weight at birth, difficulty of calving, calving to conception interval and services per conception. Sources of variation included in this model were tested by the error term. For actual MY(305-d), least squares means were analyzed using the overall model with the inclusion of the covariate actual MY(305-d) of the previous lactation. The complete mathematical model used for this analysis was:

$$Y_h = a + B_i + S_j + BS_{ij} + I_m + E_{ijl}$$

Where:

Y_h:	Estimated value of dependent variable
a:	Intercept
B_j:	Bovine somatotropin (bST) treatment effect
S_j:	Calving season
BS_{ij}:	bST by season interaction
I_m:	Covariate previous 305-d lactation MY [actual 305-d MY variable]
E_{ijl}:	Random error

The third model evaluated bST, day of biopsy (day), the two-way interaction of bST*day on the dependent variables LFAT, TAG, PC, PEPCK and MTP. Least squares means for arbitrary units of mRNA for the liver enzymes were analyzed using the overall model with the inclusion of the covariate 18s (ribosomal RNA). Sources of variation included in this model were tested by using cow nested within bST as the error term. In addition, the effect of TRT on each of the biopsy days was tested using the SCLICE option of PROC MIXED. The complete mathematical model used for this analysis was:

$$Y_h = a + B_i + D_j + BD_{ij} + Cow(B_i) + I_m + E_{ijl}$$

Where:

Y_h:	Estimated value of dependent variable
A:	Intercept
B_j:	Bovine somatotropin (bST) treatment effect
D_j:	Day
BD_{ij}:	bST by day
Cow $(BD_{ij})_k$:	Cow nested within bST by treatment
I_m:	Covariate (ribosomal RNA)
E_{ijl}:	Random error

Orthogonal contrasts were used in each of these three models to compare least squares means of variables for cows supplemented with bST during the prepartum and/or during the postpartum periods. The first contrast (A) compared means of cows supplemented with bST during any period combined versus control group (TRT I vs. II, III and IV); the second (B) compared means of cows injected during the pre- and postpartum periods versus those injected only during the prepartum or only during postpartum period (IV vs. II and III, respectively). The third (C) contrast compared means of cows injected during the prepartum versus means of cows injected during the postpartum period (II vs. III).

In addition to orthogonal contrasts to compare means of treatment groups, a set of orthogonal contrasts were conducted for the third model, which evaluated bST, day of biopsy (day), the two-way interaction of bST*day on the dependent variables LFAT, TAG, PC, PEPCK and MTP, to compare least square means of variables for each day of biopsy. The first contrast (D) compared means of cows on the first day of biopsy (d -21 prepartum) versus all other means of the prepartum period combined (day -21 vs. day +2, +14 and +28); the second (E) compared means of cows obtained on day +2 postpartum versus day +14 and +28 (day +2 vs. day +14 and +28); the third (F) set of contrast compared means of cows obtained on day +14 versus +28. Following all orthogonal contrast analyses, non-orthogonal contrasts obtained from PROC MIXED also were used to compare means between TRT groups for all variables.

Regression analyses were performed up to cubic order to describe the trends in measures during the prepartum and postpartum periods for variables when significant TRT effects were detected. Regression coefficients were obtained to plot the curves, and test of heterogeneity of regression were performed to determine whether there was evidence that regression curves were not parallel (Wilcox et al., 1990). A model was used to partition the treatment source of variation into single degrees of freedom. All variables were evaluated with calving season, cow nested in calving season and the covariate week or day to the third order polynomial as sources of variation. The complete mathematical model used for this analysis was:

$$Y_h = a + B_j + Cow(B_j)_k + A_n + E_{jk}$$

Where:

Y_h:	Estimated value of dependent variable
A:	Intercept
B_j:	calving season effect
Cow $(B_j)_k$:	Cow nested within calving season
A_n	Covariate (week or day)
E_{jk}:	Random error

Health information and reproductive performance. The statistical analyses were carried out using the FREQ, GLM and LOGISTIC procedures of SAS® (SAS Institute, 2001) to determine treatment effects on the dependent variables. Disease incidence variables (number of new cases of a disease in a treatment group during the first 60 DIM) for retained fetal membranes (RFM), metritis (MET), mastitis (MAST), digestive problems (DIG), ketosis (KETO), milk fever (MF), displaced abomasum (L/R DA), lameness (LAME) and total number of sick animals in a group (SICK), and reproductive performance variables conception rate at first service (CRFS), and pregnancy rates (PR) were calculated using a spreadsheet software program (EXCEL®, Microsoft Corp., Seattle, WA).

The diseases of high incidence (>10%), number of sick animals in a group and reproductive variables also were analyzed as categorical data to evaluate the likelihood of bST supplementation to cause/prevent disease or impair/improve reproductive function. For that, counts (frequencies) of diseased and non-diseased animals (dependent variable) in the groups supplemented with bST (independent variable), as well as the cows pregnant at first service (CRFS) or that became pregnant sometime during the subsequent lactation (PR) were compared with cows in the control group (TRT I) in a Test of Homogeneity of Proportions (Khuri, 2000). This test was carried out by the PROC FREQ procedure of SAS® (SAS Institute, 2001), and it estimated the Chi-Square (χ^2), where significant χ^2 values (≤ 0.05) indicated that the frequencies of diseased and pregnant cows differed between the groups. Because the number of cows distributed in the two calving seasons in each treatment group was insufficient to detect any statistical significance, no season effect within each TRT group was analyzed. The evaluation of the distribution of diseased and non-diseased cows between seasons was carried out with all cows combined to evaluate overall effect of calving season on disease incidence, regardless of the treatment.

Another step in the statistical analyses was to obtain odds ratios (OR) and their respective confidence intervals by using the PROC LOGISTIC procedure for the diseases of high incidence and for reproductive performance variables. A logistic regression predicts the log odds for a dependent variable as an additive function of an independent variable. The odds ratio (OR, the antilogarithm of the coefficient for an independent variable) was used as an approximate measure of relative risk. An odds ratio greater than 1 implies an increased risk of contracting a disease with increasing value of independent variable, and an odd ratios less than 1 implies a decreased risk of contracting a disease with increasing value of independent variable (Gröhn et al., 1989). Treatment effects were tested between each group of supplemented bST cows and controls, and as for the χ^2 test, OR for season effects were obtained for all cows combined, regardless treatment.

CHAPTER 4
RESULTS

Physiological Adaptations during the Prepartum Period

The variables that reflect metabolic adaptations of dairy cows during the transition period and early lactation that were evaluated in this study included body weight (BW), body condition score (BCS), and concentrations of insulin, somatotropin, insulin-like growth factor-I (IGF-I), calcium, glucose, non-esterified fatty acids (NEFA) and β-hydroxybutyrate (B-HBA) in plasma. During the prepartum period, sample collections and measurements began about 21 d prepartum and extended until day of calving for all variables evaluated. All data collected during this period were analyzed using the first statistical model, which was used to determine least squares means for all measurements, to describe overall patterns during the prepartum period, and to detect differences among treatment (TRT) groups, the two calving seasons (SEA) and the two-way interaction. The results of correlation analyses for all variables during the prepartum period are in appendix A.

Body Weight and Body Condition Score

The least squares analyses of variance and least squares means for BW and BCS during the prepartum period are in Tables 10 and 11; least squares means for each week of observation are plotted in Figures 5 and 6. No effects of TRT (P=0.6140), SEA (P=0.5669) or the two-way interaction (P=0.1645) were detected for BW. Similarly, no effects of TRT (P=0.3841) were detected for BCS, but a SEA effect was detected (P≤0.0001), and there was a trend for a two-way interaction (P≤0.1071; Table 10). Mean BW and BCS for cows on TRT I, II, III and IV were 726.7 and 3.49; 734.5 and 3.34; 739.0 and 3.47; and 751.8 kg and 3.46 pt., respectively (Table 11). With regard to orthogonal and non-orthogonal contrasts conducted, a trend for difference between TRT I and II for BCS during prepartum period was detected (P≤0.1041). Overall, the trends for changes in BW and BCS were similar during the prepartum period (Figures 5 and 6), although no significant correlation was detected between these two variables (Appendix A). Both mean BW and BCS of cows that calved during season II were greater than during season I, but difference was significant only for BCS (Table 11).

Hormones and Growth Factor

The least squares analyses of variance and the least squares means for insulin, somatotropin and IGF-I during the prepartum period are in Tables 12 and 13; least squares means for each day of observation are plotted in Figures 7 through 9. From least squares analysis of variance for insulin, no effects of TRT (P=0.5025), SEA (P=0.9184) or the two-way interaction (P=0.1341) were detected (Table 12). Mean concentrations of insulin in plasma of cows on TRT I, II, III and IV were 0.85, 0.84, 0.94 and 0.93 ng/mL (Table 13). Orthogonal and non-orthogonal contrasts did not detect significant effects or trends due to TRT or season of calving during the prepartum period (Table 13). The plot of least squares means (Figure 7) shows that variation in concentrations among samples and overall trends were similar across the four treatments.

With regard to somatotropin, results were similar to those for insulin. No TRT (P=0.1568), SEA (P=0.6628) or two-way interaction effects (P=0.6850) were detected

Table 10. Least squares analyses of variance for body weight (BW) and body condition score (BCS) of Holstein cows during the prepartum period[1].

		Body Weight[2]				Body Condition Score[2]		
Source	Df [3]	MS[3]	F[3]	Pr > F[3]	Df[3]	MS[3]	F[3]	Pr > F[3]
bST[4]	3	8467	0.60	0.6140	3	0.371	1.03	0.3841
Season[5]	1	4629	0.33	0.5669	1	13.509	37.34	0.0001
bST*Season	3	24367	1.74	0.1645	3	0.755	2.09	0.1071
Cow (bST*Season)[6]	94	13897	15.41	0.0001	94	0.361	10.92	0.0001
Wk[7]	1	14449	16.03	0.0001	1	0.151	4.57	0.0337
Wk*Wk	1	818	0.91	0.3417	1	0.001	0.03	0.8617
Error	326	901			326	0.033		

[1] From 21 d prepartum through calving.

[2] 103 multiparous Holstein cows. Body Condition Score: 1 (thin) to 5 (overconditioned) scale (Edmonson et al., 1989).

[3] df (degrees of freedom), MS (Mean square), F (F-Statistic) and Pr>F (Probability).

[4] Treatments: I (Control), II (bST Post), III (bST Pre) and IV (bST Pre/Post).

[5] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb).

[6] Error term for sources above it.

[7] Week.

Table 11. Least squares means and SEM for body weight (BW) and body condition score (BCS) of Holstein cows for treatments and season of calving during the prepartum period[1].

	Treatments					Orthogonal Comparisons			
	Control (n=26)	bST Post (n=25)	bST Pre (n=27)	bST Pre/Post (n=25)	SEM	Overall	A[3]	B[4]	C[5]
Variable						------------*P*------------			
BW, kg	726.8	734.1	739.0	751.8	13.55	NS	NS	NS	NS
BCS,pt.[2]	3.49	3.34	3.47	3.46	0.07	NS	NS	NS	NS

	Seasons[6]			
	I (n=52)	II (n=51)	SEM	Overall
BW, kg	733.7	741.6	9.58	NS
BCS, pt.[2]	3.23	3.65	0.04	**

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[1] From 21 d prepartum through calving. [2] 1 (thin) to 5 (overconditioned) scale (Edmonson et al., 1989). [3] Contrast A: I (Control) vs. II (bST Post) + III (bST Pre) + IV (bST Pre/Post). [4] Contrast B: IV (bST Pre/Post) vs. II (bST Post) + III (bST Pre). [5] Contrast C: II (bST Post) vs. III (bST Pre). [a,b] Superscripts that differ indicate significance ($P \leq 0.05$) for non-orthogonal contrasts. [6] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb). † $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$.

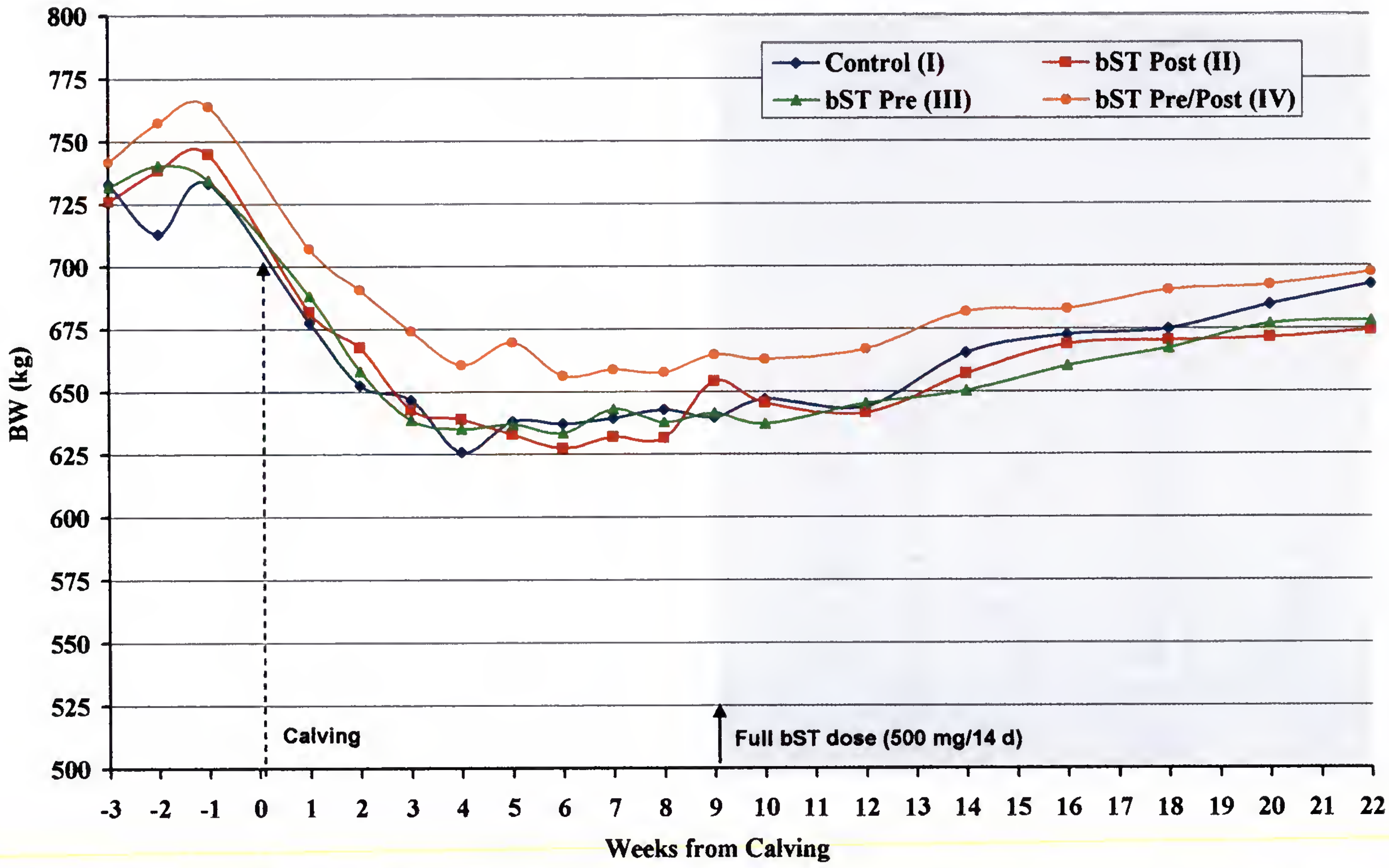

Figure 5. Least squares means depicting body weights (BW) of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. Vertical dashed line indicates calving. Shaded area represents time during which full dose of bST was supplemented to all cows.

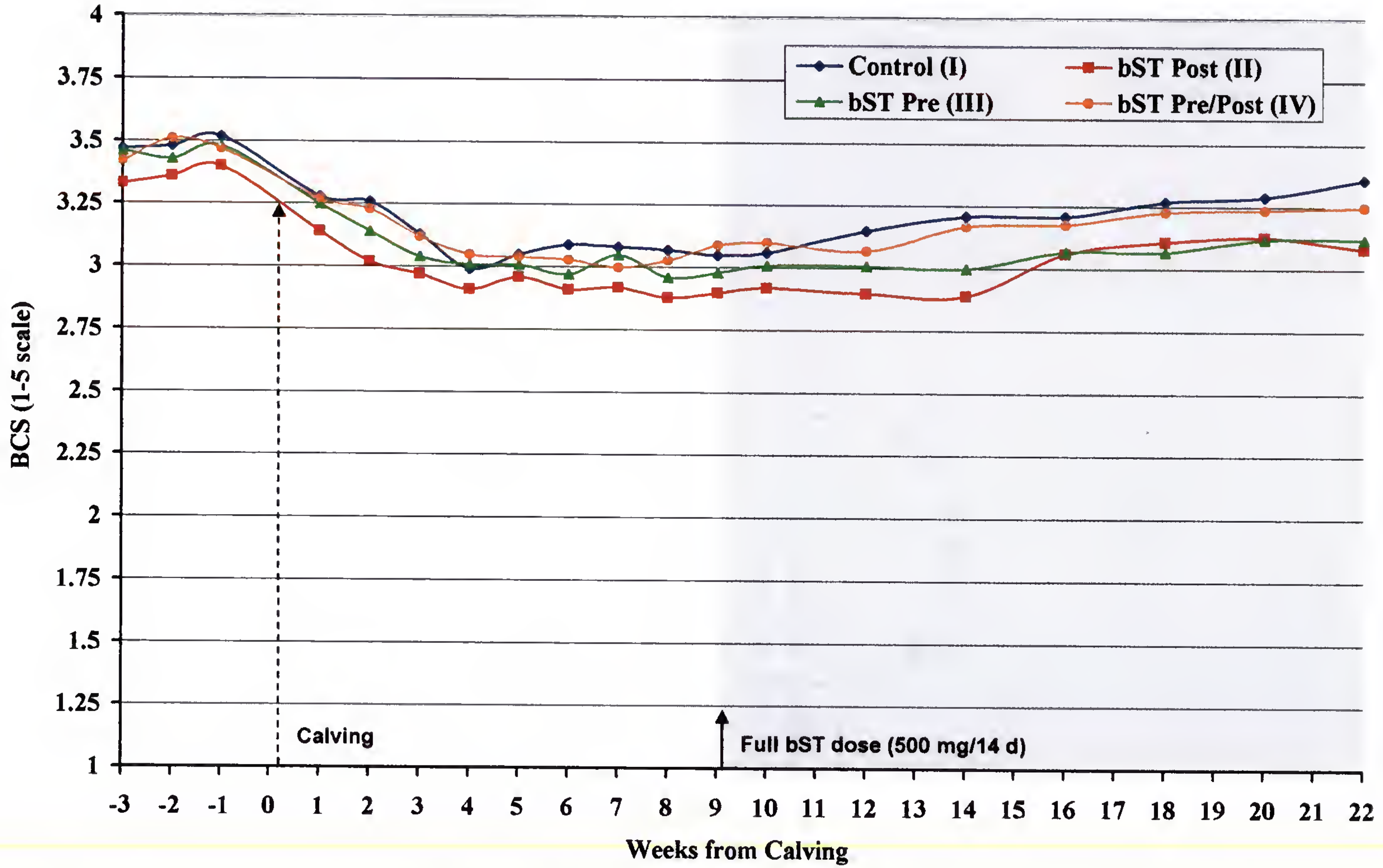

Figure 6. Least squares means depicting body condition scores (BCS) of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. Vertical dashed line indicates calving. Shaded area represents time during which full dose of bST was supplemented to all cows.

(Table 12). Mean concentrations of somatotropin in plasma of experimental cows on the four treatments were 7.34, 6.85, 7.74 and 8.00 ng/mL, respectively. Orthogonal contrasts of treatment means detected a trend for difference for contrast II vs. III ($P \leq 0.0663$). In addition, non-orthogonal contrasts conducted showed that somatotropin concentrations differed for the comparison TRT II vs. IV during prepartum period ($P \leq 0.0264$; Table 13). Overall, general trends in concentrations of somatotropin during the 3 wk prepartum were similar across treatments and for seasons of calving with no evidence of differences apparent because of bST supplementation to cows in groups III and IV.

For IGF-I, no TRT ($P=0.1482$), SEA ($P=0.1428$) or two-way interaction ($P=0.2039$) effects were detected during the prepartum period (Table 12). Mean concentrations of IGF-I in plasma of cows on the four treatments were 143.1, 152.4, 149.6 and 172.3 ng/mL, respectively. Orthogonal contrasts of means showed a trend for a difference for contrast IV vs. II and III ($P \leq 0.0659$), and subsequent non-orthogonal comparisons showed a significant difference in mean concentrations for cows in TRT groups I and IV ($P \leq 0.0284$), and a trend for difference for cows in TRT groups III and IV ($P \leq 0.0859$; Table 13). The trends for IGF-I concentrations in plasma of all groups appeared to differ, especially during wk 2 and 3 preceding calving (Figure 9). Cows on TRT III showed an increase in IGF-I concentrations in plasma during this period, whereas cows in other groups showed decreased (II) or stable concentrations (I and IV). At about 10 d before calving, a sharp decrease in plasma concentrations of the growth factor was observed in plasma of cows in all TRT groups.

Overall, figures depicting least squares means of insulin, somatotropin and IGF-I concentrations (Figure 7 through 9) showed that changes in mean concentrations of these two hormones and growth factor in plasma were similar among all TRT groups, although changes in concentrations did occur as cows approached calving. Correlation analyses among these three variables detected only a small and negative correlation between insulin and somatotropin during the prepartum period, and no other significant correlation was observed (appendix A).

Calcium and Metabolites

The least squares analyses of variance and least squares means for calcium, glucose, NEFA and B-HBA during the prepartum period are in Tables 14, 15 and 16; and least squares means for each day of observation are plotted in Figures 10 through 13. No TRT (P=0.6278), SEA (P=0.8207) or two-way interaction (P=0.3044) effects were detected for prepartum concentrations of calcium in plasma (Table 14). Mean concentrations of calcium of cows on the four treatments were similar across treatments during the prepartum period (Table 16). With regard to orthogonal and non-orthogonal contrasts of least squares means, no significant effects were detected among TRT groups during the prepartum period.

For glucose concentrations in plasma, least squares analysis of variance did not detect TRT (P=0.6886) or two-way interaction effects (P=0.3798), but there was an effect of calving season ($P \leq 0.0012$; Table 14). Least squares means for glucose for the treatment groups were 73.55, 75.56, 75.16 and 75.55 mg/dL. In addition, mean glucose concentrations in plasma of experimental cows that calved during season I were greater compared to season II (+6.3%, 72.68 vs.77.23 md/dL, Table 16). Orthogonal and non-orthogonal contrasts did not detect differences among TRT groups for glucose during the prepartum period (Table 16). The patterns of changes in concentrations of glucose

Table 12. Least squares analyses of variance for concentrations of insulin, somatotropin and insulin-like growth factor-I (IGF-I) in plasma of Holstein cows during the prepartum period[1].

	Insulin[2]				Somatotropin[2]				IGF-I[2]			
Source	Df[3]	MS[3]	F[3]	Pr > F[3]	Df[3]	MS[3]	F[3]	Pr > F[3]	Df[3]	MS[3]	F[3]	Pr > F[3]
bST[4]	3	0.622	0.79	0.5025	3	52.40	1.78	0.1568	3	33891	1.82	0.1482
Season[5]	1	0.008	0.01	0.9184	1	5.64	0.19	0.6628	1	40593	2.18	0.1428
bST*Season	3	1.500	1.90	0.1341	3	14.66	0.50	0.6850	3	29027	1.56	0.2039
Cow (bST*Season)[6]	94	0.768	4.01	0.0001	95	29.93	4.04	0.0001	95	18530	13.06	0.0001
Day	1	18.010	93.89	0.0001	1	53.94	7.29	0.0071	1	7739	5.45	0.0198
Day*Day	1	0.008	0.05	0.8299	1	0.36	0.05	0.8241	1	30096	21.21	0.0001
Day*Day*Day	1	1.861	9.71	0.0019	1	3.60	0.49	0.4857	1	35986	25.36	0.0001
Error	798	0.191			802	7.40			805	1419		

[1] From 21 d prepartum through calving.

[2] 103 multiparous Holstein cows.

[3] df (degrees of freedom), MS (Mean square), F (F-Statistic) and Pr>F (Probability).

[4] Treatments: I (Control), II (bST Post), III (bST Pre) and IV (bST Pre/Post).

[5] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb).

[6] Error term for sources above it.

Table 13. Least squares means and SEM for concentrations of insulin (INS), somatotropin (ST) and insulin-like growth factor-I (IGF-I) for treatments and season of calving in plasma of Holstein cows during the prepartum period[1].

	Treatments					Orthogonal Comparisons			
	Control (n=26)	bST Post (n=25)	bST Pre (n=27)	bST Pre/Post (n=25)	SEM	Overall	A[2]	B[3]	C[4]
Variable						----------	---------- *P*	----------	----------
INS, ng/mL	0.85	0.84	0.94	0.93	0.06	NS	NS	NS	NS
ST, ng/mL	7.34^{ac}	6.85^{ab}	7.74^{ac}	8.00^{c}	0.37	NS	NS	NS	†
IGF-1, ng/mL	143.18^{a}	152.46^{ab}	149.60^{ab}	172.39^{b}	9.39	NS	NS	†	NS

	Seasons[5]			
	I (n=52)	II (n=51)	SEM	Overall
INS, ng/mL	0.89	0.89	0.04	NS
ST, ng/mL	7.40	7.57	0.26	NS
IGF-1, ng/mL	147.46	161.35	6.65	NS

[1] From 21 d prepartum through calving. [2] Contrast A: I (Control) vs. II (bST Post) + III (bST Pre) + IV (bST Pre/Post). [3] Contrast B: IV (bST Pre/Post) vs. II (bST Post) + III (bST Pre). [4] Contrast C: II (bST Post) vs. III (bST Pre). [a,b,c] Superscripts that differ indicate significance ($P \leq 0.05$) for non-orthogonal contrasts. [5] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb). † $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$.

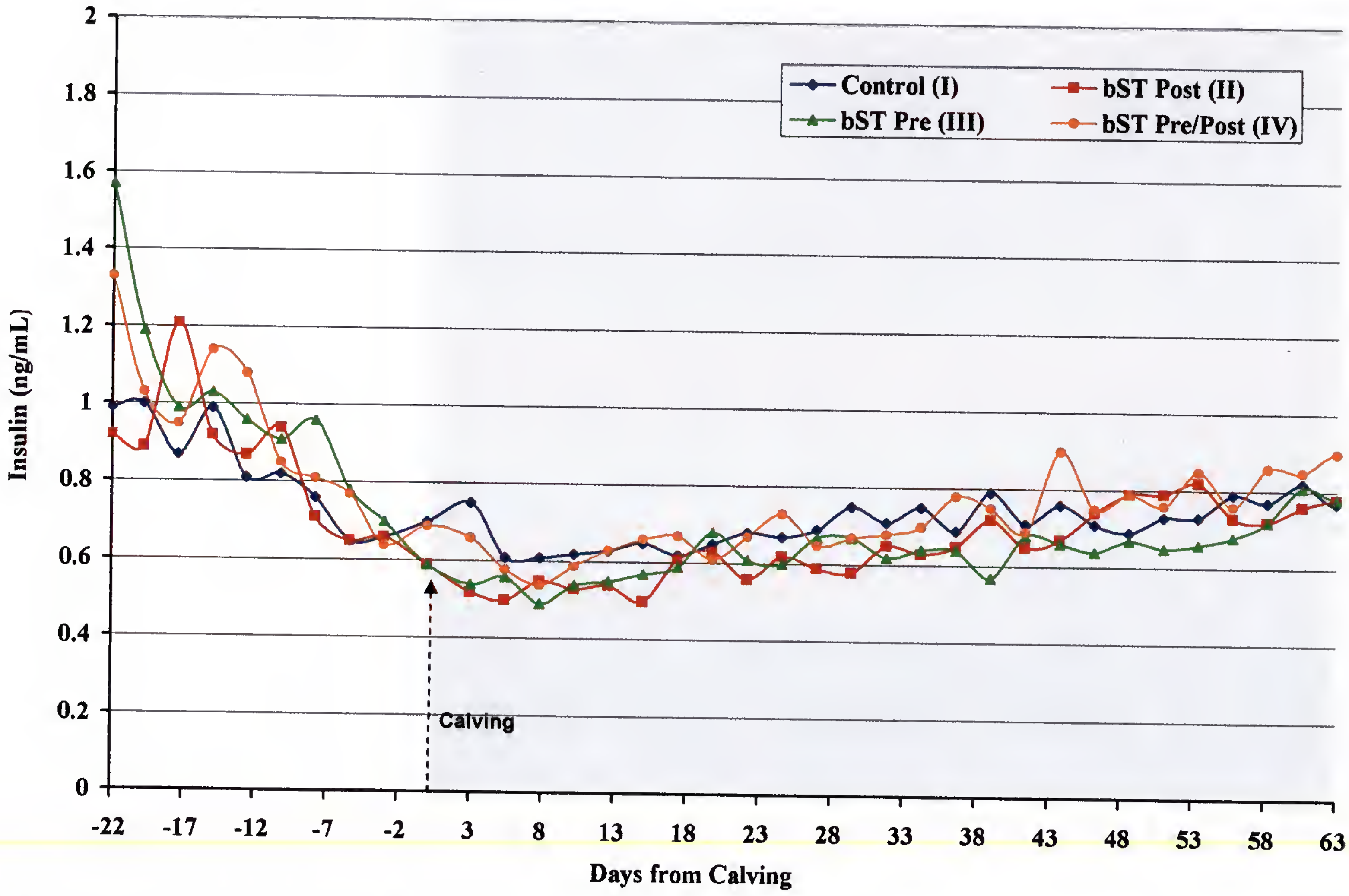

Figure 7. Least squares means depicting insulin concentrations in plasma of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. Vertical dashed line indicates calving. Shaded area represents period after calving.

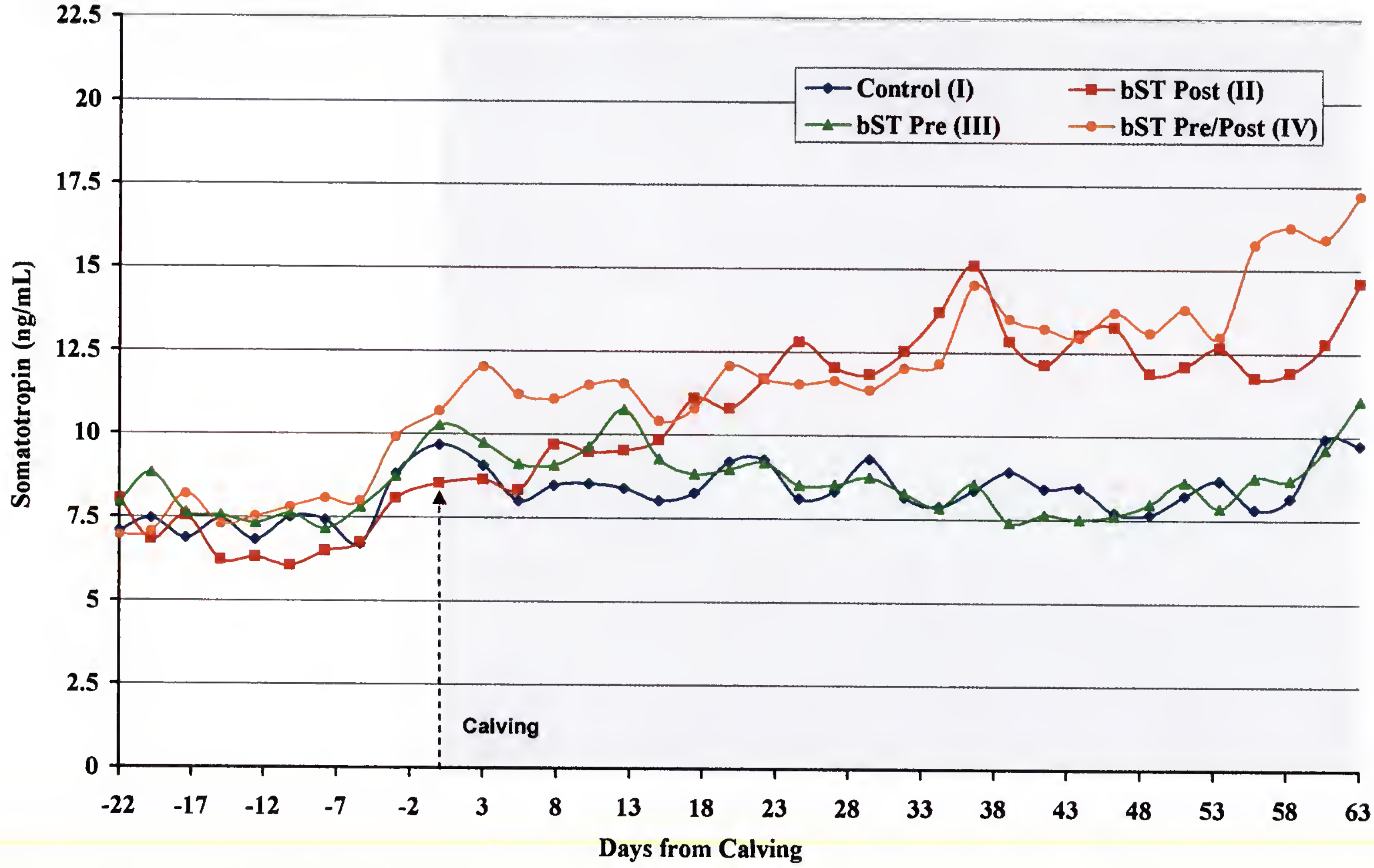

Figure 8. Least squares means depicting somatotropin concentrations in plasma of multiparous Holstein cows supplemented or not with bST during the prepartum and/or the postpartum periods. Vertical dashed line indicates calving. Shaded area represents period after calving.

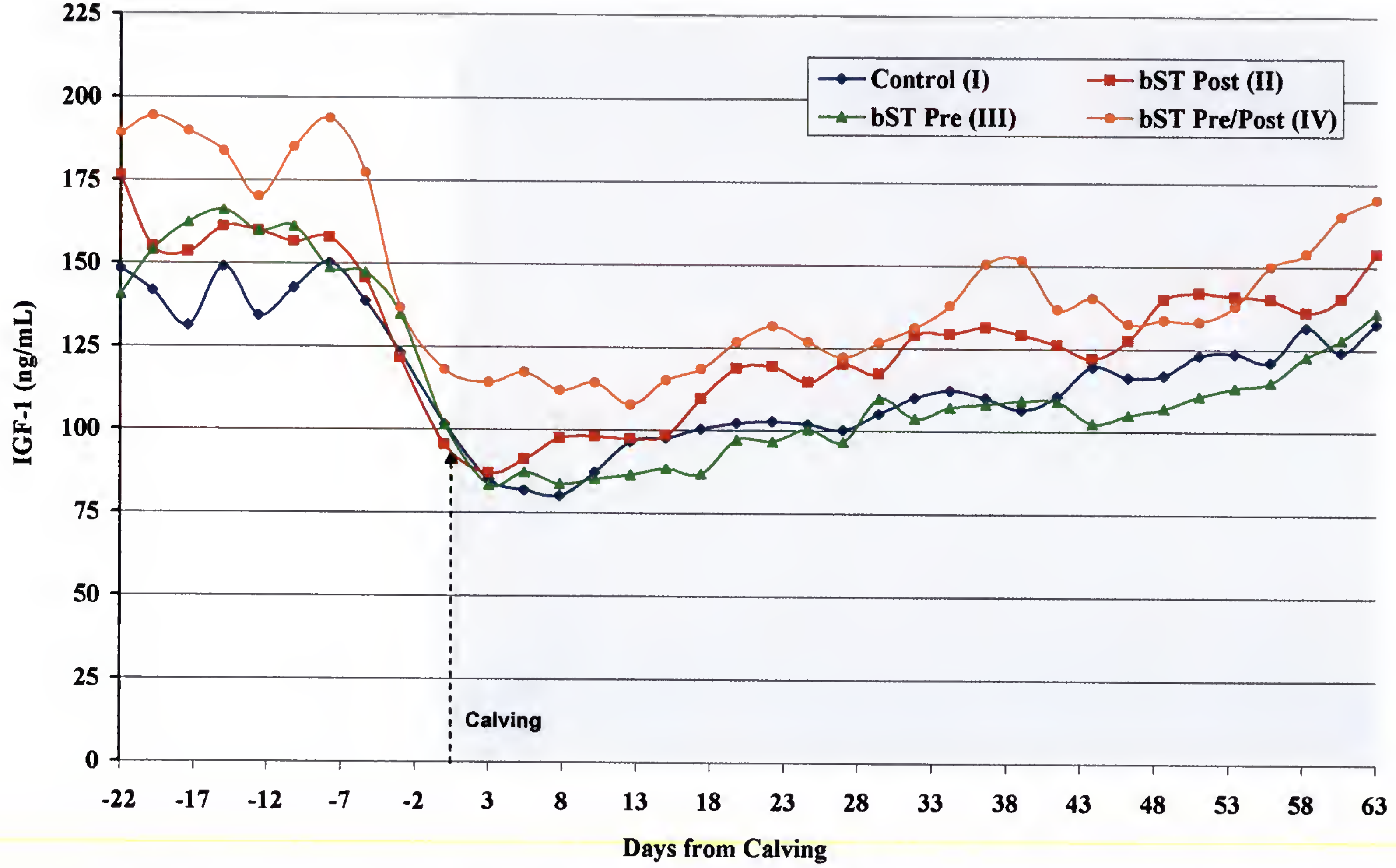

Figure 9. Least squares means depicting IGF-I concentrations in plasma of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. Vertical dashed line indicates calving. Shaded area represents period after calving.

in plasma of all experimental groups of cows were similar during the prepartum period. In general, plasma concentrations of glucose were constant up to 10 d before calving, then decreased slowly as parturition approached (Figure 10).

Results of least squares analysis of variance for concentrations of NEFA in plasma were similar to results described for glucose during the prepartum period. No TRT ($P=0.9422$) or two-way interaction effects ($P=0.4485$) were detected; however a significant SEA ($P\leq0.0013$) effect was observed (Table 15). Mean concentrations of NEFA in plasma during the prepartum were 701.5, 680.0, 671.2, and 660.0 μEq/L for the four treatments groups of cows; and during calving season I and II mean concentrations of NEFA in plasma were 597.73 and 758.68 μEq/L (Table 16). Orthogonal and non-orthogonal contrasts did not detect significant differences among least squares means of cows in the four TRT groups (Table 16). The changes in NEFA concentrations in plasma of all treatment groups during the prepartum were similar (Figure 12). In general, concentrations were constant until ~9 or 10 d prepartum, then increased markedly as calving approached. With regard to B-HBA, from least squares analysis of variance, no TRT ($P=0.3258$), SEA ($P=0.6994$) or two-way interaction ($P=0.8471$) effects were detected during the prepartum period (Table 15). The mean concentrations of B-HBA in plasma of cows on TRT I, II, III and IV were 4.81, 4.77, 4.50 and 4.19 mg/dL, respectively; and orthogonal and non-orthogonal contrasts analysis did not detect any differences among means of the TRT groups (Table 16). The trends in changes in concentrations as calving approached across treatments for B-HBA were similar to those seen for NEFA concentrations in plasma (Figure 13). Concentrations of B-HBA were constant until about 1 wk before calving, then began to increase. Correlation analysis indicated that small but significant correlations were detected between calcium and other metabolites, between metabolites and between the mineral/metabolites and hormones/growth factor; these are summarized in Appendix A.

Physiological Adaptations during the Postpartum Period

During the postpartum period, measurements of body weight and body condition scores following calving were recorded through 150 DIM. For concentrations of insulin, somatotropin, insulin-like growth factor-I and glucose in plasma, measurements began at calving and were measured in samples collected through 63 DIM. However, for calcium, NEFA and B-HBA their concentrations in plasma only were measured in samples collected through 28 DIM. Respective data collected for each variable were analyzed using the first statistical model; which was used to obtain least squares means, to describe overall patterns of changes in concentrations during the postpartum period, and to detect differences among treatment (TRT) groups, the two calving seasons (SEA), any two-way interactions. Results of correlation analyses among all variables during the postpartum period are in Appendix B.

Body Weight and Body Condition Score

For body weights (BW) and body condition scores (BCS), data were analyzed over each of the three time periods: from calving (day 0) through 63 DIM, from 64 through 150 DIM (early to middle lactation), and from 0 to 150 DIM. Least squares analyses of variance and least squares means for BW and BCS for each of the time periods of the postpartum phase are in Tables 17, 18, 19 and 20. Also, least squares means for each week of observation are plotted in Figures 5 and 6.

Table 14. Least squares analyses of variance for concentrations of calcium and glucose in plasma of Holstein cows during the prepartum period[1].

		Calcium[2]				Glucose[2]		
Source	Df[3]	MS[3]	F[3]	Pr > F[3]	Df[3]	MS[3]	F[3]	Pr > F[3]
bST[4]	3	4.25	0.58	0.6278	3	193.58	0.49	0.6886
Season[5]	1	0.37	0.05	0.8207	1	4361.02	11.08	0.0012
bST*Season	3	8.96	1.23	0.3044	3	408.04	1.04	0.3798
Cow (bST*Season)[6]	95	7.40	15.54	0.0001	95	387.76	4.02	0.0001
Day	1	0.13	0.28	0.5939	1	338.53	3.51	0.0613
Day*Day	1	2.11	4.45	0.0353	1	139.87	1.45	0.2287
Day*Day*Day	1	2.02	4.26	0.0394	1	1548.57	16.07	0.0001
Error	709	0.47			803	96.36		

[1] From 21 d prepartum through calving.

[2] 103 multiparous Holstein cows.

[3] df (degrees of freedom), MS (Mean square), F (F-Statistic) and Pr>F (Probability).

[4] Treatments: I (Control), II (bST Post), III (bST Pre) and IV (bST Pre/Post).

[5] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb).

[6] Error term for sources above it.

Table 15. Least squares analyses of variance for concentrations of non-esterified fatty acids and β-hydroxybutyrate in plasma of Holstein cows during the prepartum period[1].

		Non-Esterified Fatty Acids[2]				β-Hydroxybutyrate[2]		
Source	Df[3]	MS[3]	F[3]	Pr > F[3]	Df[3]	MS[3]	F[3]	Pr > F[3]
bST[4]	3	60926.71	0.13	0.9422	3	15.945	1.17	0.3258
Season[5]	1	5131310.28	10.92	0.0013	1	2.046	0.15	0.6994
bST*Season	3	418795.72	0.89	0.4485	3	3.679	0.27	0.8471
Cow (bST*Season)[6]	95	417118.47	6.07	0.0001	95	13.304	4.91	0.0001
Day	1	5487715.65	79.89	0.0001	1	81.656	30.14	0.0001
Day*Day	1	1437592.80	20.93	0.0001	1	53.264	19.66	0.0001
Day*Day*Day	1	794244.82	11.56	0.0007	1	1.793	0.66	0.4161
Error	717	68691.90			720	2.709		

[1] From 21 d prepartum through calving.

[2] 103 multiparous Holstein cows.

[3] df (degrees of freedom), MS (Mean square), F (F-Statistic) and Pr>F (Probability).

[4] Treatments: I (Control), II (bST Post), III (bST Pre) and IV (bST Pre/Post).

[5] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb).

[6] Error term for sources above it.

Table 16. Least squares means and SEM for concentrations of calcium (Ca), glucose (GLC), non-esterified fatty acids (NEFA) and β-hydroxybutyrate (B-HBA) for treatments and season of calving during the prepartum period[1].

	Treatments					Orthogonal Comparisons			
	Control (n=26)	bST Post (n=25)	bST Pre (n=27)	bST Pre/Post (n=25)	SEM	Overall	A[2]	B[3]	C[4]
Variable						----------P----------			
Ca, mg/dL	9.18	9.49	9.21	9.19	0.19	NS	NS	NS	NS
GLC, mg/dL	73.55	75.56	75.16	75.55	1.36	NS	NS	NS	NS
NEFA, µEq/L	701.52	680.06	671.21	660.03	48.77	NS	NS	NS	NS
B-HBA, mg/dL	4.81	4.77	4.50	4.19	0.26	NS	NS	NS	NS

	Seasons[5]			
	I (n=52)	II (n=51)	SEM	Overall
Ca, mg/dL	9.29	9.25	0.13	NS
GLC, mg/dL	72.68	77.23	0.96	**
NEFA, µEq/L	597.73	758.68	34.49	**
B-HBA, mg/dL	7.72	12.30	0.96	**

[1] From 21 d prepartum through calving. [2] Contrast A: I (Control) vs. II (bST Post) + III (bST Pre) + IV (bST Pre/Post). [3] Contrast B: IV (bST Pre/Post) vs. II (bST Post) + III (bST Pre). [4] Contrast C: II (bST Post) vs. III (bST Pre). [a,b] Superscripts that differ indicate significance ($P \leq 0.05$) for non-orthogonal contrasts. [5] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb). † $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$.

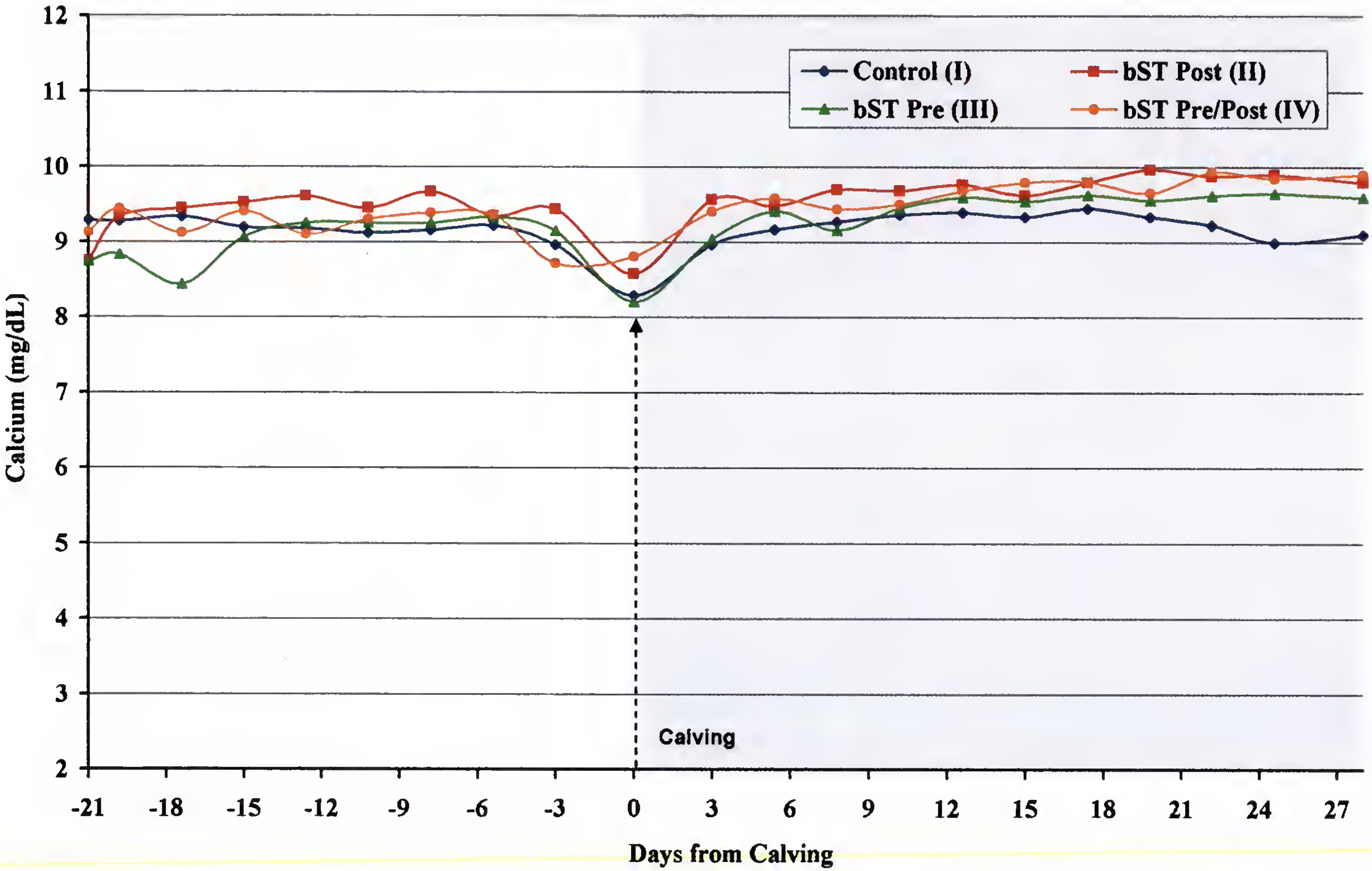

Figure 10. Least squares means depicting calcium concentrations in plasma of multiparous Holstein cows supplemented or not with bST during the prepartum and/or during the postpartum periods. Vertical dashed line indicates calving. Shaded area represents period after calving

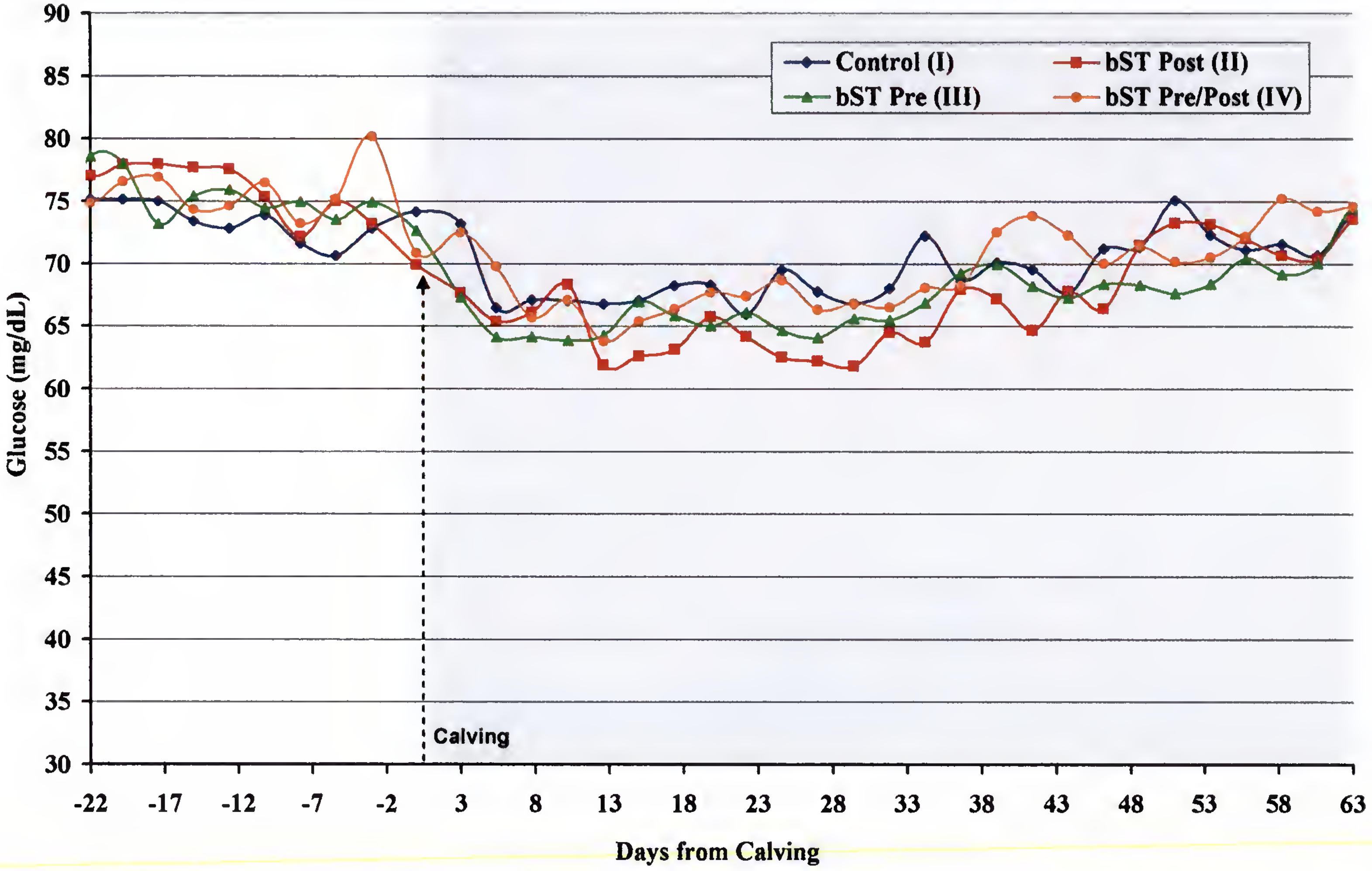

Figure 11. Least squares means depicting glucose concentrations in plasma of multiparous Holstein cows supplemented or not with bST during the prepartum and/or during the postpartum periods. Vertical dashed line indicates calving. Shaded area represents period after calving.

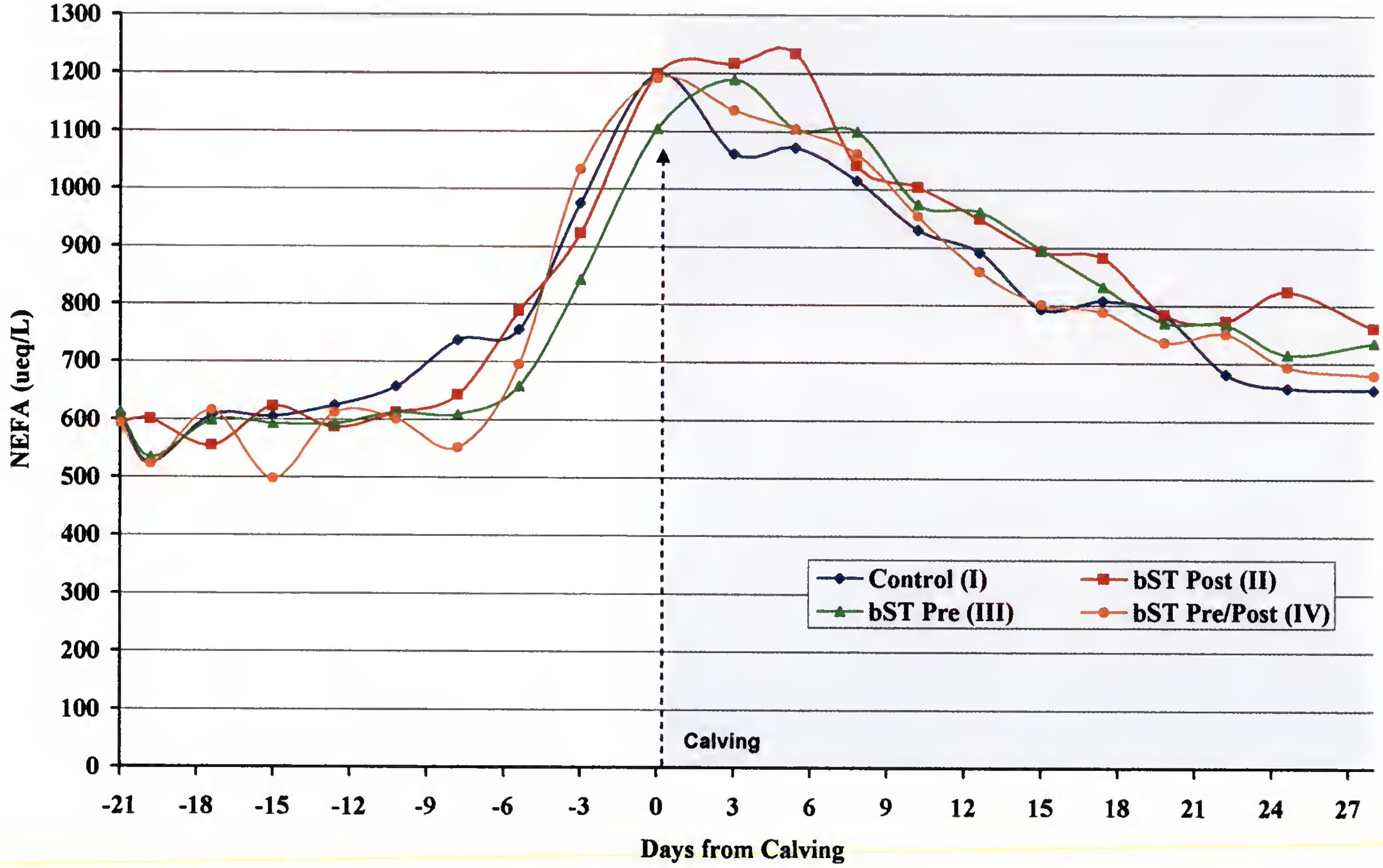

Figure 12. Least squares means depicting NEFA concentrations in plasma of multiparous Holstein cows supplemented or not with bST during the prepartum and/or during the postpartum periods. Vertical dashed line indicates calving. Shaded area represents period after calving.

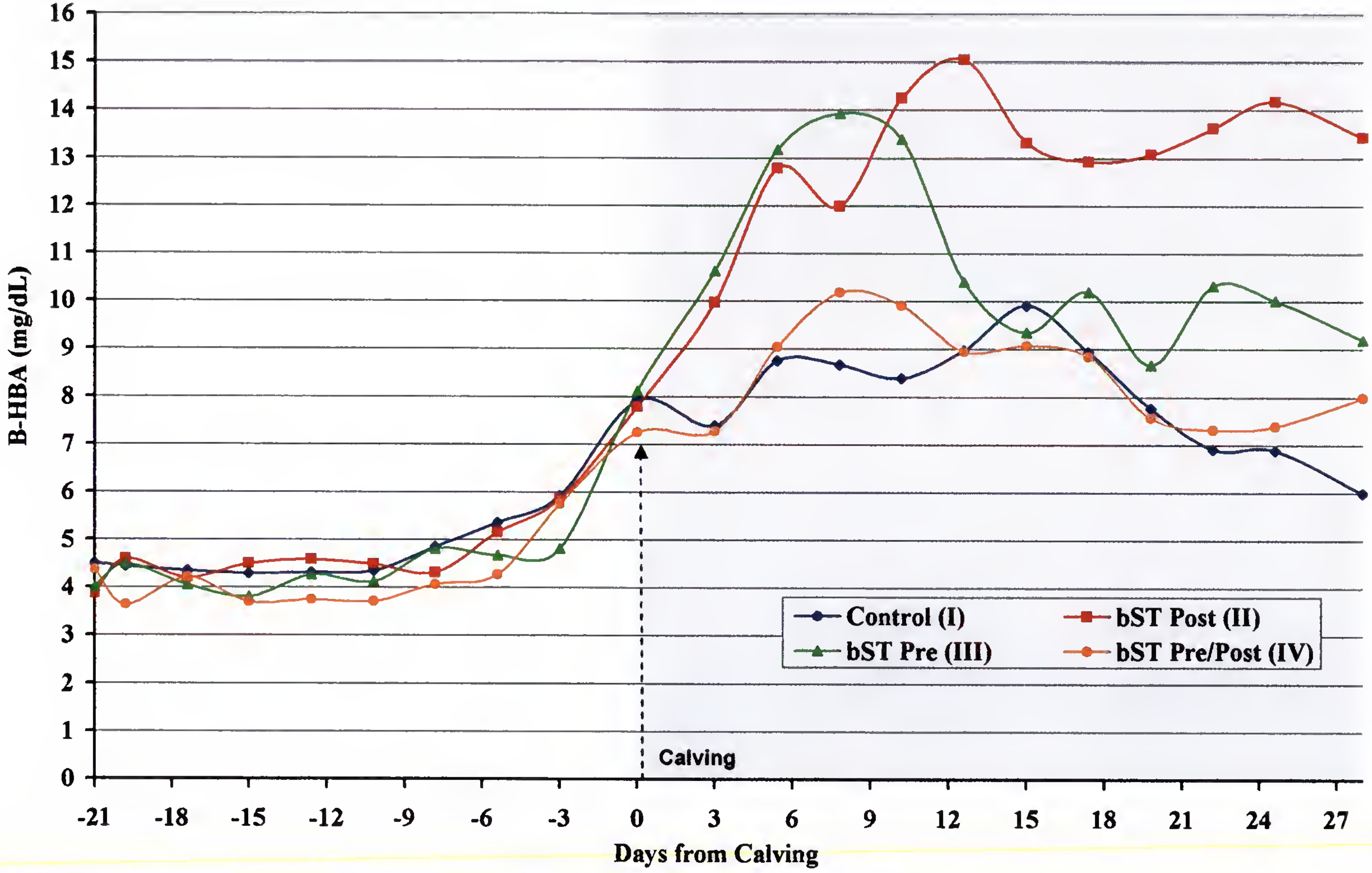

Figure 13. Least square means depicting β-hydroxybutyrate (B-HBA) concentrations in plasma of multiparous Holstein cows supplemented or not with bST during the prepartum and/or during the postpartum periods. Vertical dashed line indicates calving. Shaded area represents period after calving.

Least squares analysis of variance for the time period 0-63 DIM detected no effects of TRT (P=0.2834), SEA (P=0.1490) or the two-way interaction (P=0.1856) for BW. Similarly, no TRT (P=0.3118), SEA (P=0.7771) or interaction (P=0.4319) effects were observed for BCS (Table 17). Mean BW and BCS for each of the four treatment groups of cows are 648.4 and 3.12; 647.1 and 2.97; 649.5 and 3.06; and 673.9 kg and 3.10 pt., respectively (Table 20). For BW, analyses by orthogonal contrasts indicated a trend of differences in means for the contrast IV vs. II and III ($P \leq 0.0612$). Subsequent non-orthogonal contrasts of least squares means during the 0-63 DIM also detected a trend for the comparisons TRT I vs. IV ($P \leq 0.1057$), and TRT II vs. IV ($P \leq 0.0912$) to differ. For BCS, the only trend detected was for comparison TRT I vs. II ($P \leq 0.0709$; Table 20). Indeed, Figure 5 shows that TRT IV cows maintained a higher BW than cows on TRT II and III, and Figure 6 shows that TRT II cows had the lowest BCS during the period, especially when compared to TRT I cows.

Similar least squares analyses of variance were conducted using data for BW and BCS collected during the time period 64-150 DIM; these analyses for BW detected no effects due to TRT (P=0.4275), SEA (P=0.4448) or two-way interaction (P=0.3526). Similarly, no TRT (P=0.1463) or interaction (P=0.4233) effects were observed for BCS, but there was a trend for least squares means for the two seasons to differ ($P \leq 0.0994$; Table 18). Mean BCS during the two calving seasons were 3.15 and 3.03 pt, respectively. Mean BW and BCS for cows on the four treatments were 670.9 and 3.20; 663.7 and 3.00; 654.4 and 3.01; and 680.5 kg and 3.16 pt., respectively (Table 20). Also, orthogonal contrasts of least squares means for BCS showed that there was a trend for contrasts TRT I vs. II, III and IV ($P \leq 0.0896$) and IV vs. II and III ($P \leq 0.0955$) to differ. Non-orthogonal contrasts also detected a difference in means for BCS for TRT I vs. II ($P \leq 0.0510$) and I vs. III ($P \leq 0.0711$); least squares means are in Table 20. Figure 6 clearly shows that cows on TRT I had greater BCS than cows on TRT II and III during 64-150 DIM, but the mean score of cows in the control group (TRT I) did not differ from those of TRT group IV.

For the overall postpartum period (0-150 DIM), analyses of BW data detected no effect of TRT ($P=0.2865$), SEA ($P=0.2058$) or two-way interaction ($P=0.1855$). Similarly, no TRT ($P=0.1551$), SEA ($P=0.4711$) or interaction ($P=0.3594$) were observed for BCS (Table 19). However, orthogonal contrasts detected differences between means of BW for TRT IV vs. TRT II and III ($P \leq 0.0550$), and subsequent non-orthogonal comparisons of least squares means indicated that mean BW of cows for both TRT II and III cows tended to differ from those of TRT IV ($P \leq 0.1063$; $P \leq 0.0839$, respectively). For BCS, orthogonal contrasts detected no significant TRT effects for any of the comparisons. However, non-orthogonal contrasts of least squares means did detect a difference between TRT I vs. II means for BCS ($P \leq 0.0382$), and there was a trend for BCS of cows on TRT II vs. IV ($P \leq 0.0907$; Table 20) to differ. In addition to TRT effects, there was a strong overall positive correlation between BW and BCS during the postpartum period (Appendix B).

Because differences in least squares means for TRT were detected for 0-150 DIM data by orthogonal and non-orthogonal contrasts (TRT IV vs. II and III for BW, and between TRT I vs. II for BCS), regression analyses for both BW and BCS were performed for this time period. Test of heterogeneity of regression also were conducted using the general model to partition the sources of variation for treatments into single degrees of freedom. All variables were evaluated using a mathematical model that included calving season using cow(season) as the error term, and the covariate day to the third order polynomial also was included as source of variation. The cubic regressions for BW and BCS were significant (curvilinearity, $P \leq 0.0001$). Regression coefficients and the intercepts for BW and BCS for each of the four treatments are in Tables 21 and 22 and the regression curves are plotted in Figures 14 and 15.

The regression curves for BW clearly showed that differences existed between TRT IV vs. TRT II and III; the latter two treatments had similar regression curves during the first half of the lactation (0-150 DIM). Also, cows on TRT I better maintained BCS than cows on TRT II and III for the period beyond 11-12 wk of lactation, but no apparent differences between TRT I and TRT IV were observed during 0-150 DIM. Heterogeneity of regression for BW indicated that regression curves were not parallel, but for BCS there was no evidence to indicate that they were not parallel (Appendix C); similar interpretation was obtained from evaluation of the least squares means for these variables plotted over this same time period (Figures 6 and 14).

Hormones and Growth Factor

The least squares analyses of variance and least squares means for insulin, somatotropin (ST) and IGF-I during the postpartum period are in Tables 23 and 24; and least squares means for each day of observation are plotted in Figures 7 through 9. For least squares mean concentrations of insulin in plasma during the postpartum period, no TRT ($P=0.3055$) or two-way interaction ($P=0.6558$) effects were detected, but a trend for SEA ($P \leq 0.0906$) effect was observed (Table 23). Mean insulin concentrations in plasma of cows on TRT I, II, III and IV were 0.72, 0.66, 0.65 and 0.74 ng/mL, respectively. During calving seasons I and II, mean insulin concentrations in plasma were 0.72 and

Table 17. Least squares analyses of variance for body weight (BW) and body condition score (BCS) of Holstein cows from 0-63 DIM.

		Body Weight[1]				Body Condition Score[1]		
Source	Df[2]	MS[2]	F[2]	Pr > F[2]	Df[2]	MS[2]	F[2]	Pr > F[2]
bST[3]	3	39289	1.30	0.2834	3	1.12	1.21	0.3118
Season[4]	1	64583	2.13	0.1490	1	0.07	0.08	0.7771
bST*Season	3	49882	1.64	0.1856	3	0.86	0.94	0.4319
Cow (bST*Season)[5]	95	30575	43.02	0.0001	95	0.93	22.19	0.0001
Wk[6]	1	164971	232.10	0.0001	1	5.70	135.90	0.0001
Wk*Wk	1	122724	172.67	0.0001	1	1.94	46.41	0.0001
Wk*Wk*Wk	1	7834.98	11.02	0.0009	1	0.23	5.65	0.0176
Error	895	718.71			895	0.04		

[1] 103 multiparous Holstein cows milked 3x/d. Body Condition Score: 1 (thin) to 5 (overconditioned) scale (Edmonson et al., 1989).

[2] df (degrees of freedom), MS (Mean square), F (F-Statistic) and Pr>F (Probability).

[3] Treatments: I (Control), II (bST Post), III (bST Pre) and IV (bST Pre/Post).

[4] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb).

[5] Error term for sources above it.

[6] Week.

Table 18. Least squares analyses of variance for body weight (BW) and body condition score (BCS) of Holstein cows from 64-150 DIM.

	Body Weight[1]				Body Condition Score[1]			
Source	Df[2]	MS[2]	F[2]	Pr > F[2]	Df[2]	MS[2]	F[2]	Pr > F[2]
bST[3]	3	18664.82	0.93	0.4275	3	1.52	1.82	0.1463
Season[4]	1	11828.69	0.59	0.4448	1	2.36	2.83	0.0994
bST*Season	3	22011.08	1.10	0.3526	3	0.78	0.94	0.4233
Cow (bST*Season)[5]	95	20004.22	52.21	0.0001	95	0.84	28.11	0.0001
Wk[6]	1	133763.85	349.12	0.0001	1	2.75	92.24	0.0001
Wk*Wk	1	2431.71	6.35	0.0120	1	0.01	0.48	0.4872
Wk*Wk*Wk	1	6062.54	0.02	0.8999	1	0.09	3.23	0.0728
Error	614	382.52			614	0.03		

[1] 102 multiparous Holstein cows milked 3x/d. Body Condition Score: 1 (thin) to 5 (overconditioned) scale (Edmonson et al., 1989).

[2] df (degrees of freedom), MS (Mean square), F (F-Statistic) and Pr>F (Probability).

[3] Treatments: I (Control), II (bST Post), III (bST Pre) and IV (bST Pre/Post).

[4] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb).

[5] Error term for sources above it.

[6] Week.

Table 19. Least squares analyses of variance for body weight (BW) and body condition score (BCS) of Holstein cows from 0-150 DIM.

	Body Weight[1]				Body Condition Score[1]			
Source	Df[2]	MS[2]	F[2]	Pr > F[2]	Df[2]	MS[2]	F[2]	Pr > F[2]
bST[3]	3	58856.20	1.25	0.2865	3	2.71	1.73	0.1551
Season[4]	1	75855.51	1.61	0.2058	1	0.79	0.50	0.4711
bST*Season	3	77500.13	1.64	0.1855	3	1.67	1.07	0.3594
Cow (bST*Season)[5]	95	46844.01	62.03	0.0001	95	1.57	33.03	0.0001
Wk[6]	1	78885.08	104.46	0.0001	1	0.20	4.38	0.0364
Wk*Wk	1	286364.40	379.22	0.0001	1	7.65	160.26	0.0001
Wk*Wk*Wk	1	142223.81	188.34	0.0001	1	2.96	62.08	0.0001
Error	1719	755.15			1719	0.04		

[1] 103 (from 0-63 DIM) or 102 (from 64-150 DIM) multiparous Holstein cows milked 3x/d. Body Condition Score: 1 (thin) to 5 (overconditioned) scale (Edmonson et al., 1989).

[2] df (degrees of freedom), MS (Mean square), F (F-Statistic) and Pr>F (Probability).

[3] Treatments: I (Control), II (bST Post), III (bST Pre) and IV (bST Pre/Post).

[4] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb).

[5] Error term for sources above it.

[6] Week.

Table 20. Least squares means and SEM for body weight (BW) and body condition score (BCS) of Holstein cows for treatments and season of calving during various time periods postpartum.

	Treatments					Orthogonal Comparisons			
	Control (n=26)	bST Post (n=25)	bST Pre (n=27)	bST Pre/Post (n=25)	SEM	Overall	A^5	B^6	C^7
Variable						P			
BW, kg^1	648.4	647.1	649.4	673.9	11.06	NS	NS	†	NS
BW, kg^2	670.9	663.7	654.4	680.5	11.58	NS	NS	NS	NS
BW, kg^3	658.7	653.6	652.3	678.3	10.44	NS	NS	**	NS
BCS,pt.^{1,4}	3.12	2.97	3.06	3.10	0.06	NS	NS	NS	NS
BCS,pt.^{2,4}	3.20^{a}	3.00^{bc}	3.01^{bc}	3.16^{ac}	0.07	NS	†	†	NS
BCS,pt.^{3,4}	3.16^{a}	2.98^{b}	3.05^{ab}	3.12^{ab}	0.05	NS	NS	NS	NS

	Seasons^8			
	I (n=52)	II (n=51)	SEM	Overall
BW, kg^1	662.8	646.6	7.82	NS
BW, kg^2	671.9	662.9	8.19	NS
BW, kg^3	667.5	654.0	7.78	NS
BCS,pt.^{1,4}	3.05	3.07	0.04	NS
BCS,pt.^{2,4}	3.15	3.03	0.04	†
BCS,pt.^{3,4}	3.10	3.05	0.04	NS

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[1] 103 multiparous Holstein cows milked 3x/day from 0-63 DIM. [2] 102 multiparous Holstein cows milked 3x/day from 64-150 DIM. [3] multiparous Holstein cows milked 3x/day from 0-150 DIM. [4] Body Condition Score: 1 (thin) to 5 (overconditioned) scale (Edmonson et al., 1989). [5] Contrast A: I (Control) vs. II (bST Post) + III (bST Pre) + IV (bST Pre/Post). [6] Contrast B: IV (bST Pre/Post) vs. II (bST Post) + III (bST Pre). [7] Contrast C: II (bST Post) vs. III (bST Pre). [a,b,c] Superscripts that differ indicate significance ($P \leq 0.05$) for non-orthogonal contrasts. [8] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb). $^{\dagger} P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$.

Table 21. Cubic regression coefficients that describe treatment effects on body weight (BW) of Holstein cows from 0-150 DIM[1].

Treatment	β0[2]	β1[2]	± SEM	β2[2]	± SEM	β3[2]	± SEM
Control (I)	675.1	-13.04942	4.73261	1.26708	0.48188	-0.02880	0.01386
bST Post (II)	689.2	-18.76062	4.85409	1.79855	0.50461	-0.04506	0.01474
bST Pre (III)	686.8	-16.25180	4.10721	1.49525	0.42012	-0.03532	0.01210
bST Pre/Post (IV)	716.2	-18.38248	4.43911	1.74818	0.45140	-0.04450	0.01294

[1] 103 (from 0-63 DIM) or 102 (from 64-150 DIM) multiparous Holstein cows milked 3x/d.

[2] β0 (Intercept), β1 (wk), β2 (wk*wk) and β3 (wk*wk*wk). Wk stands for week.

R^2 = 0.0875 (Control), 0.0610 (bST Post), 0.0739 (bST Pre) and 0.0526 (bST Pre/Post).

Mathematical model used: bST, season, bST*season, using cow (bST*season) as error term, with week to the third order as covariate.

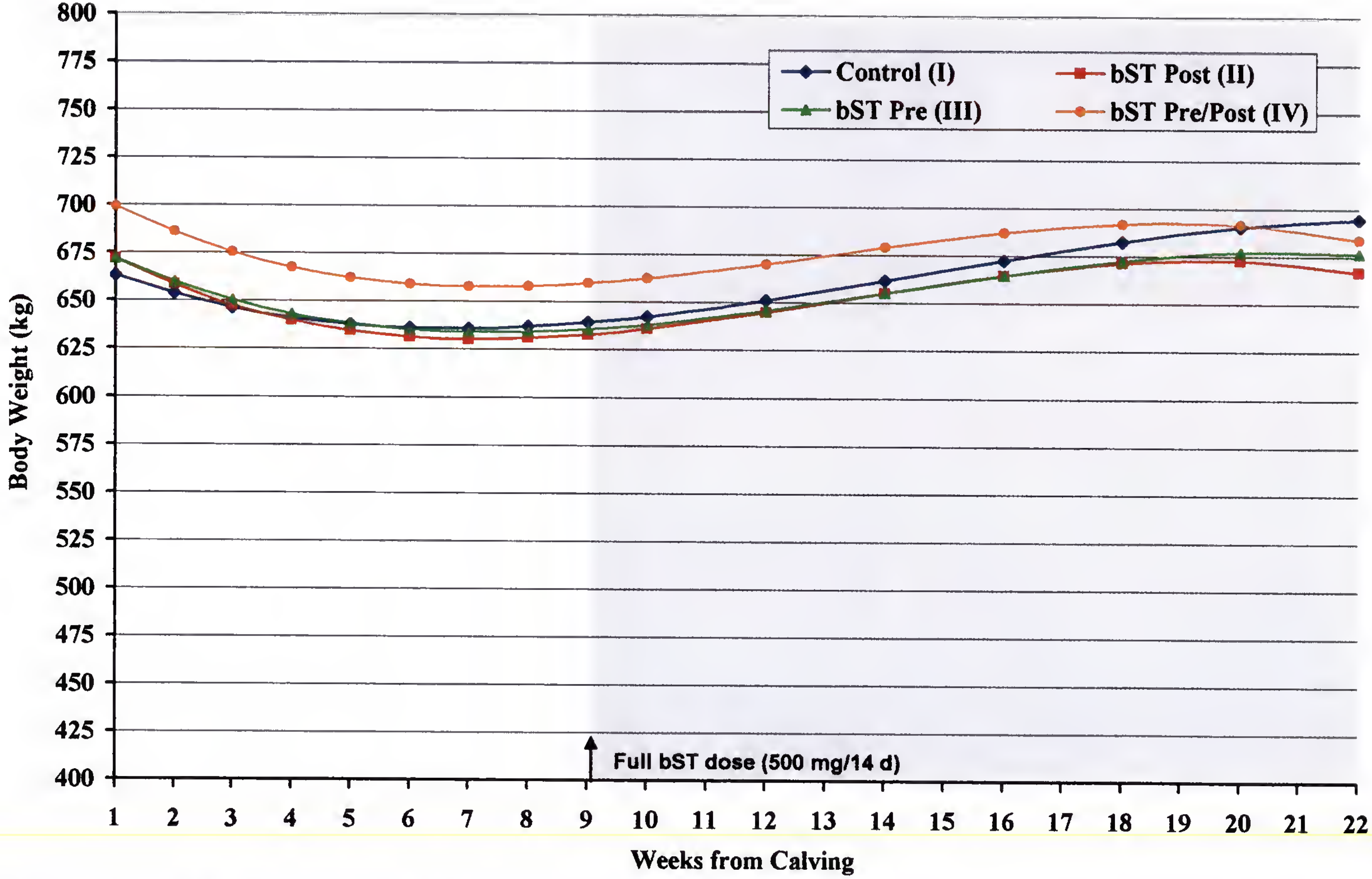

Figure 14. Cubic regression curves depicting body weight changes of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. Model: bST, season, bST*season effect, using cow(bST*season) as error, with wk to the third order as covariate. Shaded area represents time during which full dose of bST was supplemented to all cows.

Table 22. Cubic regression coefficients that describe treatment effects on body condition score (BCS) of Holstein cows from 0-150 DIM[1].

Treatment	β0[2]	β1[2]	± SEM	β2[2]	± SEM	β3[2]	± SEM
Control (I)	3.36157	-0.10055	0.03565	0.00950	0.00363	-0.00022641	0.00010441
bST Post (II)	3.20284	-0.09382	0.02432	0.00831	0.00253	-0.00019675	0.00007387
bST Pre (III)	3.24207	-0.07277	0.02316	0.00625	0.00237	-0.00014637	0.00006824
bST Pre/Post (IV)	3.23339	-0.09616	0.02752	0.00944	0.00280	-0.00024509	0.00008024

[1] 103 (from 0-63 DIM) or 102 (from 64-150 DIM) multiparous Holstein cows milked 3x/d.

[2] β0 (Intercept), β1 (wk), β2 (wk*wk) and β3 (wk*wk*wk). Wk stands for week.

R^2 = 0.0466 (Control), 0.0593 (bST Post), 0.0336 (bST Pre) and 0.0389 (bST Pre/Post).

Mathematical model used: bST, season, bST*season, using cow (bST*season) as error term, with week to the third order as covariate.

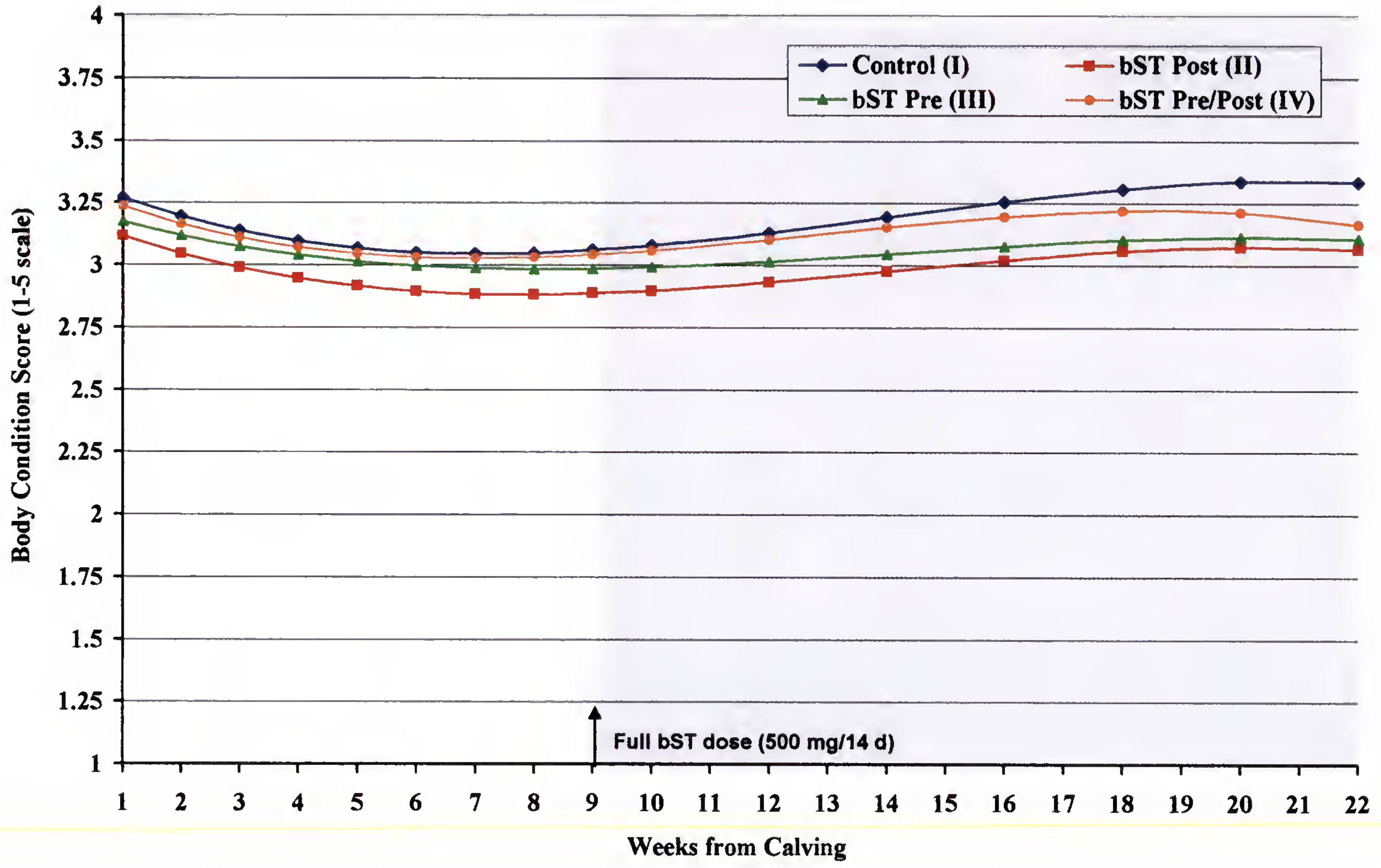

Figure 15. Cubic regression curves depicting body condition score of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. Model: bST, season, bST*season effect, using cow(bST*season) as error, with wk to the third order as covariate. Shaded area represents time during which full dose of bST was supplemented to all cows.

0.66 ng/mL, respectively (Table 24). Orthogonal contrasts of least squares treatment means detected a trend of a TRT effect for the contrast IV vs. II and III ($P \leq 0.0866$). Subsequent comparisons of least squares means by non-orthogonal contrasts indicated that TRT II and TRT III did not differ from TRT IV (Table 24).

For concentrations of somatotropin, both TRT ($P \leq 0.0001$) and SEA ($P \leq 0.0104$) effects were observed and there was a tendency for a two-way interaction ($P \leq 0.0710$; Table 23). Mean concentrations of somatotropin in plasma of cows on the four treatments were 8.68, 12.32, 9.27 and 13.42 ng/mL, respectively (Table 24). All orthogonal contrasts of treatment means were significant; contrast A [$P \leq 0.0001$], B [$P \leq 0.0001$] and C [$P \leq 0.0001$]. Non-orthogonal contrasts indicated that the least squares means of concentrations of somatotropin for TRT I and III differed ($P \leq 0.0001$) from those of TRT II and IV (Table 24).

With regard to concentrations of IGF-I in plasma during the postpartum period, significant TRT ($P \leq 0.0284$) and a trend for SEA ($P \leq 0.0778$) effects were detected, but not for the two-way interaction ($P=0.6365$; Table 23). Mean concentrations of IGF-I in plasma of cows on TRT I, II, III and IV during the postpartum period are in Table 24. Orthogonal contrasts indicated that least squares means for TRT IV vs. II and III differed ($P \leq 0.0466$), and there was a trend for II vs. III means to differ ($P \leq 0.0977$). Non-orthogonal comparisons of treatment means showed that IGF-I concentrations in cows on TRT IV differed from TRT I ($P \leq 0.0153$) and also differed from III ($P \leq 0.0107$; Table 24). Correlation analyses detected positive correlations between IGF-I and insulin, and also for IGF-I and ST during the postpartum period (Appendix B).

Because a treatment effect on mean concentrations of both somatotropin and IGF-I were detected during the postpartum period (0-63 DIM), regression analyses were performed. Test for heterogeneity of regression were completed using the general least squares analysis of variance model to partition the sources of variation for treatments into single degrees of freedom. All variables were evaluated with calving season, cow nested in calving season and the covariate day to the third order polynomial as sources of variation, using cow(season) as error term for sources above it in the mathematical model. Regression coefficients and the intercepts of the somatotropin and IGF-I regressions for day for each of the four treatments are in Tables 25 and 26 and the regression curves are plotted in Figures 16 and 17.

The cubic regressions for somatotropin and IGF-I were significant (curvilinearity, $P \leq 0.01$). The polynomial curves for somatotropin demonstrated that groups of cows that were supplemented with bST during the postpartum period (II and IV) had greater concentrations of somatotropin in plasma than controls (Figure 16). In addition, shapes of the regression curves also differed among TRT groups (Appendix C). The polynomial curves for concentrations of IGF-I demonstrated that cows on TRT IV, which received bST supplementation during both prepartum and postpartum periods, maintained greater concentrations in plasma than non-supplemented controls (TRT I) and those cows that were supplemented only prepartum (TRT III, Figure 17). As shown for the somatotropin regression curves, heterogeneity of regression of the IGF-I curves indicated that they were not parallel (Appendix C).

Table 23. Least squares analyses of variance for concentrations of insulin, somatotropin and insulin-like growth factor-I (IGF-I) in plasma of Holstein cows during 0-63 DIM.

	Insulin[1]				Somatotropin[1]				IGF-I[1]			
Source	Df[2]	MS[2]	F[2]	Pr > F[2]	Df[2]	MS[2]	F[2]	Pr > F[2]	Df[2]	MS[2]	F[2]	Pr > F[2]
bST[3]	3	1.41	1.22	0.3055	3	3988.0	22.72	0.0001	3	156745	3.16	0.0284
Season[4]	1	3.36	2.92	0.0906	1	1200.4	6.84	0.0104	1	157937	3.18	0.0778
bST*Season	3	0.62	0.54	0.6558	3	424.3	2.42	0.0710	3	28287	0.57	0.6365
Cow (bST*Season)[5]	95	1.11	19.54	0.0001	95	171.0	9.86	0.0001	95	46803	44.90	0.0001
Day	1	18.01	316.40	0.0001	1	4802.7	276.66	0.0001	1	1105837	1060.9	0.0001
Day*Day	1	0.23	4.11	0.0427	1	771.4	44.44	0.0001	1	322	0.31	0.5780
Day*Day*Day	1	0.11	2.04	0.1531	1	155.4	8.95	0.0028	1	8256	7.92	0.0049
Error	2938	0.05			2936	17.3			2946	1042		

[1] 103 multiparous Holstein cows milked 3x/d.

[2] df (degrees of freedom), MS (Mean square), F (F-Statistic) and Pr>F (Probability).

[3] Treatments: I (Control), II (bST Post), III (bST Pre) and IV (bST Pre/Post).

[4] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb).

[5] Error term for sources above it.

Table 24. Least squares means and SEM for concentrations of insulin (INS), somatotropin (ST) and insulin-like growth factor-I (IGF-I) in plasma of Holstein cows for treatments and season of calving during 0-63 DIM.

	Treatments					Orthogonal Comparisons			
	Control (n=26)	bST Post (n=25)	bST Pre (n=27)	bST Pre/Post (n=25)	SEM	Overall	A[1]	B[2]	C[3]
Variable						----------P----------			
INS, ng/mL	0.72	0.66	0.65	0.74	0.03	NS	NS	†	NS
ST, ng/mL	8.68^{a}	12.32^{b}	9.27^{a}	13.42^{b}	0.47	**	**	**	**
IGF-1, ng/mL	109.63^{a}	127.44^{ac}	108.38^{a}	138.07^{bc}	8.08	*	NS	*	†

	Seasons[4]			
	I (n=52)	II (n=51)	SEM	Overall
INS, ng/mL	0.72	0.66	0.02	†
ST, ng/mL	10.29	11.56	0.33	*
IGF-1, ng/mL	128.10	113.66	5.71	†

[1] Contrast A: I (Control) vs. II (bST Post) + III (bST Pre) + IV (bST Pre/Post). [2] Contrast B: IV (bST Pre/Post) vs. II (bST Post) + III (bST Pre). [3] Contrast C: II (bST Post) vs. III (bST Pre). [a,b,c] Superscripts that differ indicate significance ($P \leq 0.05$) for non-orthogonal contrasts. [4] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb). † $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$

Table 25. Cubic regression coefficients that describe treatment effects on concentration of somatotropin in plasma of Holstein cows from 0-63 DIM [1].

Treatment	$\beta0^2$	$\beta1^2$	± SEM	$\beta2^2$	± SEM	$\beta3^2$	± SEM
Control (I)	4.24493	0.67745	0.31435	-0.03362	0.01350	0.00052331	0.00018045
bST Post (II)	0.23577	1.26525	0.49642	-0.04806	0.02155	0.00067834	0.00029171
bST Pre (III)	8.90478	0.26559	0.31662	-0.02435	0.01406	0.00050798	0.00019410
bST Pre/Post (IV)	15.60441	-0.084766	0.44297	0.04389	0.01912	-0.00054426	0.00025715

[1] 103 multiparous Holstein cows milked 3x/d.

[2] $\beta0$ (Intercept), $\beta1$ (day), $\beta2$ (day*day) and $\beta3$ (day*day*day).

$R^2 = 0.0408$ (Control), 0.1249 (bST Post), 0.0743 (bST Pre) and 0.1373 (bST Pre/Post).

Mathematical model used: bST, season, bST*season, using cow (bST*season) as error term, with day to the third order as covariate.

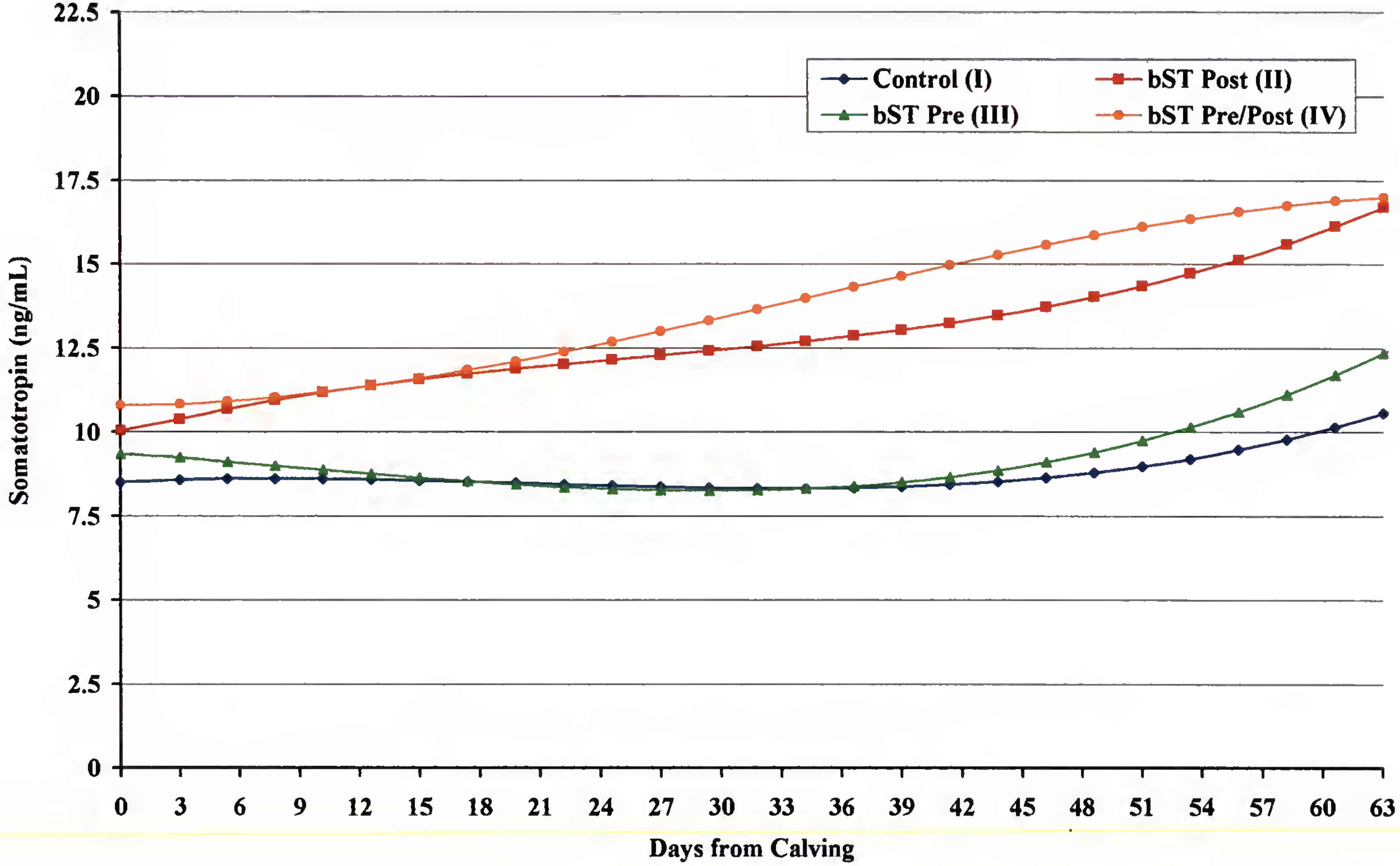

Figure 16. Cubic regression curves depicting somatotropin concentrations in plasma of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. bST, season, bST*season effect, using cow(bST*season) as error, with day to the third order as covariate.

Table 26. Cubic regression coefficients that describe treatment effects on concentrations of IGF-I in plasma of Holstein cows from 0-63 DIM[1].

Treatment	β0[2]	β1[2]	± SEM	β2[2]	± SEM	β3[2]	± SEM
Control (I)	161.68	-11.32904	3.73857	0.56364	0.16055	-0.00760	0.00215
bST Post (II)	88.54	0.08900	5.48153	0.07902	0.23792	-0.00088613	0.00322
bST Pre (III)	91.55	-1.35330	3.65184	0.11538	0.16218	-0.00136	0.00224
bST Pre/Post (IV)	44.25	8.24359	4.26456	-0.27818	0.18405	0.00398	0.00247

[1] 103 multiparous Holstein cows milked 3x/d.

[2] β0 (Intercept), β1 (day), β2 (day*day) and β3 (day*day*day).

R^2 = 0.0832 (Control), 0.0915 (bST Post), 0.0968 (bST Pre) and 0.1463 (bST Pre/Post).

Mathematical model used: bST, season, bST*season, using cow (bST*season) as error term, with day to the third order as covariate.

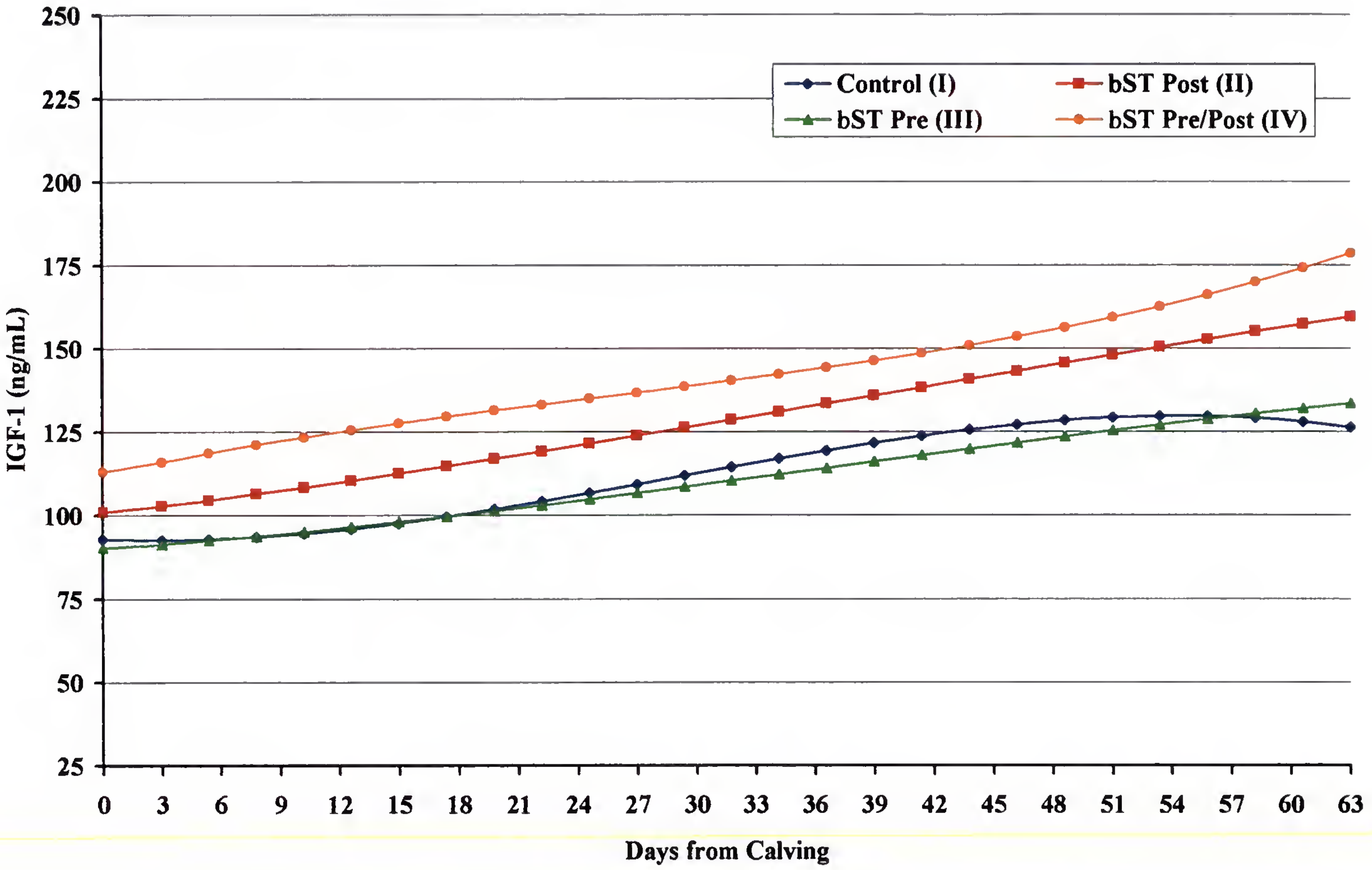

Figure 17. Cubic regression curves depicting IGF-I concentrations in plasma of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. Model: bST, season, bST*season effect, using cow(bST*season) as error, with day to the third order as covariate.

Calcium and Metabolites

Least squares analysis of variance for glucose was completed using data that was collected during the time period 0 to 63 DIM. For calcium, non-esterified fatty acids (NEFA) and β-hydroxybutyrate (B-HBA), analyses were completed using data collected during 0 to 28 DIM. The least squares analyses of variance and least squares means for glucose, calcium, NEFA and B-HBA during the postpartum period are in Tables 27, 28 and 29, and least squares means for each day of observation are plotted in Figures 10 through 13. Correlation analyses for these variables are in Appendix B.

For glucose, no TRT ($P=0.3371$), SEA ($P=0.9421$) or two-way interaction ($P=0.9398$) effects were detected (Table 27). Mean concentrations of glucose in plasma during the postpartum period for the four groups of cows (I, II, III and IV) were 70.03, 68.10, 68.03 and 70.37 mg/dL, respectively. Orthogonal contrasts of least squares means detected a tendency for TRT IV vs. II and III to differ ($P\leq 0.1099$), but subsequent non-orthogonal contrast analysis did not detect any TRT effects among groups (Table 29).

From least squares analysis of variance for calcium, no TRT ($P=0.3907$), SEA ($P=0.4459$) or two-way interaction ($P=0.4705$) effects were detected during the postpartum period (Table 27). Mean concentrations of Ca in plasma of cows of the four treatment groups were 9.17, 9.62, 9.37 and 9.55 mg/dL, respectively. Using orthogonal and non-orthogonal contrasts of least squares treatment means, no significant effects were detected among TRT groups (Table 29).

Least squares analysis of variance for postpartum concentrations of NEFA did not detect TRT ($P=0.8949$) or two-way interaction effects ($P=0.5679$), but SEA effects were evident ($P\leq 0.0001$; Table 28). Mean concentrations of NEFA in plasma during the postpartum period are in Table 29. Cows that calved during the cooler months of the year (season II, November-February) had greater mean concentrations of NEFA in plasma than in those cows that calved during the warmer months (season I); least squares mean concentrations of NEFA in plasma were 796.22 and 1049.40 µEq/L for the two seasons (Table 29). Orthogonal and non-orthogonal contrasts of least squares means did not detect differences in mean concentrations NEFA in cows of the four TRT groups (Table 29).

For B-HBA, least squares analysis of variance detected a trend due to TRT ($P \leq 0.0736$), as well as a SEA effect ($P \leq 0.0012$), but there was no interaction ($P=0.6655$) between SEA and TRT (Table 28). Mean concentrations of B-HBA in plasma of cows on TRT I, II, III and IV were 8.33, 12.68, 10.77 and 8.27 mg/dL, respectively. With regard to the effect of calving season on mean concentrations of B-HBA, cows that calved during season I had lower concentrations of B-HBA in plasma than those that calved during season II (7.72 vs. 12.30 mg/dL; Table 29). Orthogonal and non-orthogonal contrasts only detected significance for the contrast TRT IV vs. II and III ($P \leq 0.0448$). Non-orthogonal comparisons also indicated that mean concentrations of B-HBA in TRT II cows differed from those of TRT I ($P \leq 0.0277$) and IV cows ($P \leq 0.0275$; Table 29).

Because TRT effects were detected for concentrations of B-HBA during the postpartum period (0-28 DIM), regression analyses also were performed. Test for heterogeneity of regression was completed using the general least squares analysis of variance model to partition the sources of variation for treatments into single degrees of freedom. The variable B-HBA was evaluated with calving season, cow nested in calving season and the covariate day to the third order polynomial as sources of variation, using cow(season) as error term for sources above it in the mathematical model. Regression coefficients are in Table 30 and regression curves depicting changes in B-HBA are in Figure 18. The overall cubic regression was significant (curvilinearity, $P<0.01$), and the individual curves clearly showed that during the postpartum period differences existed between TRT II and TRT IV, both of which were supplemented during the postpartum period. Curve for TRT II cows also differed from the two curves for the groups of cows not supplemented postpartum (TRT I and III). Clearly, a difference was seen early in the lactation (d 4-6) for cows supplemented only during prepartum and difference increased as the lactation progressed being greatest on the last sampling day (d 28), whereas mean concentrations declined slightly in the three other groups of cows (I, III and IV).

Correlation analyses conducted for concentrations of glucose, NEFA and B-HBA in plasma during the postpartum period showed that glucose was negatively correlated with NEFA and B-HBA during the period. In addition, there was a strong positive correlation between B-HBA and NEFA in plasma (Appendix B).

Liver Metabolism of Lipids and Carbohydrates

The variables evaluated during the transition period of dairy cows that reflect liver metabolism of lipids and carbohydrates during this phase were percentage of total fat (LFAT) and percentage of triacylglycerol (TAG) in liver on a wet basis, and mRNA abundance (gene expression) of the key enzymes *pyruvate carboxylase* (PC), *phosphoenolpyruvate carboxykinase* (PEPCK) and *microsomal triacylglycerol transfer protein-1* (MTP) adjusted for 18s (ribosomal) arbitrary units values. All variables were measured in liver samples obtained at d 21 prepartum, and at d +2, +14 and +28

Table 27. Least squares analyses of variance for concentrations of glucose and calcium in plasma of Holstein cows during postpartum period[1].

	Glucose[2]				Calcium[3]			
Source	Df[4]	MS[4]	F[4]	Pr > F[4]	Df[4]	MS[4]	F[4]	Pr > F[4]
bST[5]	3	1168.37	1.14	0.3371	3	10.24	1.01	0.3907
Season[6]	1	5.43	0.01	0.9421	1	5.92	0.59	0.4459
bST*Season	3	137.02	0.13	0.9398	3	8.59	0.85	0.4705
Cow (bST*Season)[7]	95	1036.70	10.31	0.0001	95	9.38	20.08	0.0001
Day	1	17918.08	178.27	0.0001	1	75.46	161.47	0.0001
Day*Day	1	5311.66	52.85	0.0001	1	5.73	12.27	0.0005
Day*Day*Day	1	255.95	2.55	0.1107	1	2.87	6.15	0.0133
Error	2947	100.51			904	0.46		

[1] 103 multiparous Holstein cows.

[2] From 0 to 63 DIM.

[3] From 0 to 28 DIM.

[4] df (degrees of freedom), MS (Mean square), F (F-Statistic) and Pr>F (Probability).

[5] Treatments: I (Control), II (bST Post), III (bST Pre) and IV (bST Pre/Post).

[6] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb).

[7] Error term for sources above it.

Table 28. Least squares analyses of variance for concentrations of non-esterified fatty acids and β-hydroxybutyrate in plasma of Holstein cows during postpartum period[1].

	Non-Esterified Fatty Acids[2]				β-Hydroxybutyrate[2]			
Source	Df[3]	MS[3]	F[3]	Pr > F[3]	Df[3]	MS[3]	F[3]	Pr > F[3]
bST[4]	3	229686.31	0.20	0.8949	3	1324.33	2.39	0.0736
Season[5]	1	18954350.24	16.66	0.0001	1	6183.83	11.16	0.0012
bST*Season	3	770972.56	0.68	0.5679	3	291.53	0.53	0.6655
Cow (bST*Season)[6]	95	1043396.90	12.27	0.0001	95	552.99	13.32	0.0001
Day	1	31358827.50	368.90	0.0001	1	6.00	0.14	0.7038
Day*Day	1	2244145.71	26.40	0.0001	1	5.20	0.13	0.7234
Day*Day*Day	1	199719.52	2.35	0.1256	1	154.41	3.72	0.0540
Error	1095	85006.70			1091	41.51		

[1] From 0 to 28 DIM.

[2] 103 multiparous Holstein cows.

[3] df (degrees of freedom), MS (Mean square), F (F-Statistic) and Pr>F (Probability).

[4] Treatments: I (Control), II (bST Post), III (bST Pre) and IV (bST Pre/Post).

[5] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb).

[6] Error term for sources above it.

Table 29. Least squares means and SEM for concentrations of glucose (GLC), calcium (Ca), non-esterified fatty acids (NEFA) and β-hydroxybutyrate (B-HBA) for treatments and season of calving during the postpartum period.

	Treatments					Orthogonal Comparisons			
	Control (n=26)	bST Post (n=25)	bST Pre (n=27)	bST Pre/Post (n=25)	SEM	Overall	A^3	B^4	C^5
Variable						----------P----------			
GLC, mg/dL^1	70.03	68.10	68.03	70.37	1.15	NS	NS	†	NS
Ca, mg/dL^2	9.17	9.62	9.37	9.55	0.20	NS	NS	NS	NS
NEFA, μEq/L^2	912.1	961.6	922.0	895.4	61.84	NS	NS	NS	NS
B-HBA, mg/dL^2	8.33^a	12.68^b	10.77^{ab}	8.27^a	1.36	†	NS	*	NS

	Seasons^6			
	I (n=52)	II (n=51)	SEM	Overall
GLC, mg/dL^1	69.17	69.09	0.81	NS
Ca, mg/dL^2	9.50	9.35	0.14	NS
NEFA, μEq/L^2	796.22	1049.40	43.74	**
B-HBA, mg/dL^2	7.72	12.30	0.96	**

[1] From 0 to 63 DIM. [2] From 0 to 28 DIM. [3] Contrast A: I (Control) vs. II (bST Post) + III (bST Pre) + IV (bST Pre/Post). [4] Contrast B: IV (bST Pre/Post) vs. II (bST Post) + III (bST Pre). [5] Contrast C: II (bST Post) vs. III (bST Pre). [a,b] Superscripts that differ indicate significance ($P \leq 0.05$) for non-orthogonal contrasts. [6] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb). † $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$.

Table 30. Cubic regression coefficients that describe treatment effects on concentrations of β-hydroxybutyrate (B-HBA) in plasma of Holstein cows from 0-28 DIM [1].

Treatment	β0[2]	β1[2]	± SEM	β2[2]	± SEM	β3[2]	± SEM
Control (I)	16.99	-1.65703	2.17494	0.10252	0.14446	-0.00212	0.00305
bST Post (II)	17.02	-1.64193	5.22413	0.09927	0.33352	-0.00070069	0.00683
bST Pre (III)	27.30	-2.03055	4.42936	0.07747	0.30735	-0.00122	0.00682
bST Pre/Post (IV)	-4.86	2.18772	2.01621	-0.10046	0.12843	0.00112	0.00261

[1] 103 multiparous Holstein cows milked 3x/d.

[2] β0 (Intercept), β1 (day), β2 (day*day) and β3 (day*day*day).

R^2 = 0.0070 (Control), 0.0889 (bST Post), 0.0389 (bST Pre) and 0.0322 (bST Pre/Post).

Mathematical model used: bST, season, bST*season, using cow (bST*season) as error term, with day to the third order as covariate.

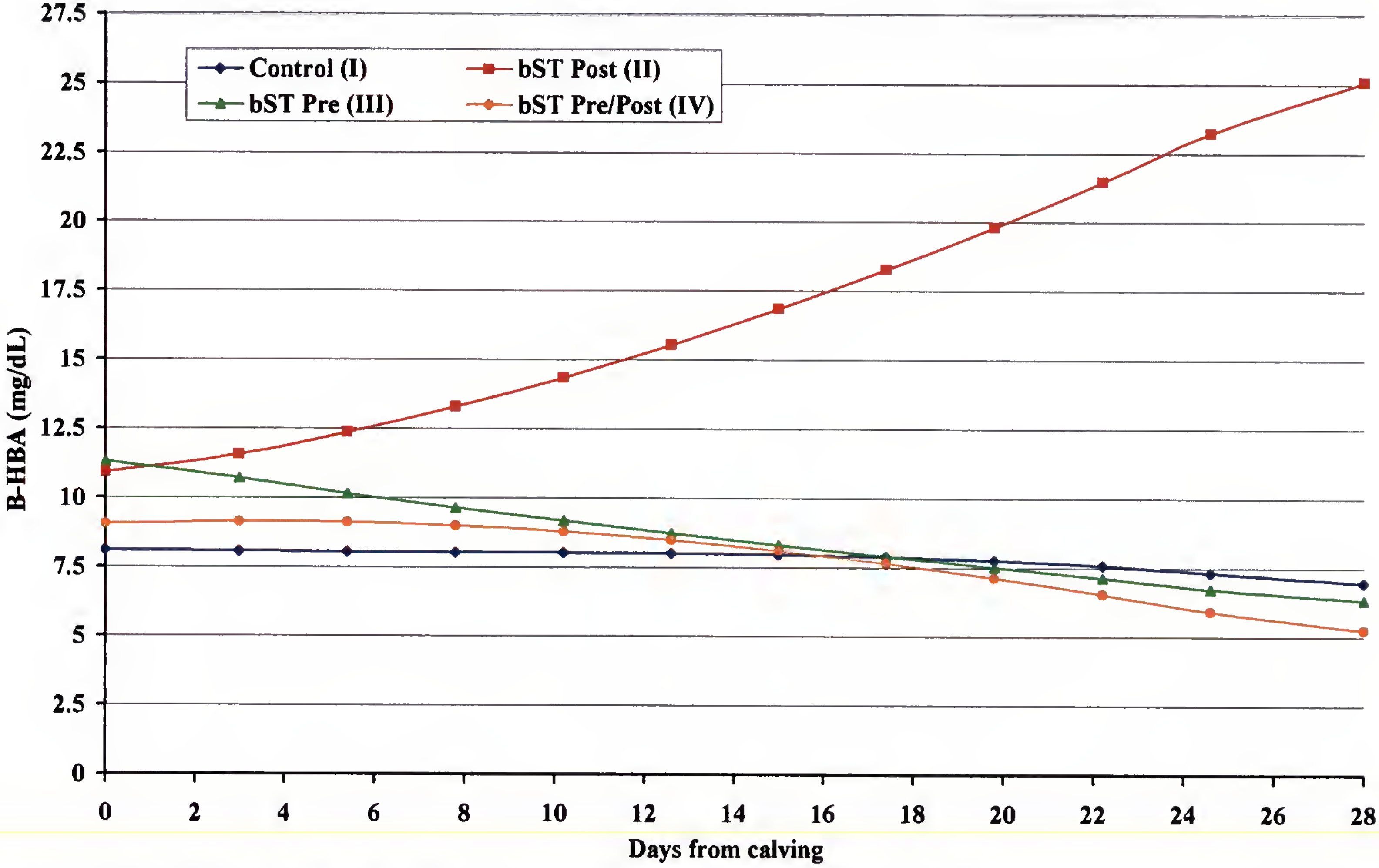

Figure 18. Cubic regression curves depicting β-hydroxybutyrate (B-HBA) concentrations in plasma of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. Model: bST, season, bST*season using cow(bST*season) as error, with day to the third order as covariate.

postpartum. All data, regardless of sample day (prepartum or postpartum), were analyzed using the third statistical model. This model evaluated effects of treatment (TRT, bST), day of biopsy (DAY) and the two-way interaction on the dependent variables. The least squares analyses of variance and least squares means for LFAT, TAG, and mRNA of enzymes PC, PEPCK and MTP are in Tables 31 through 36; and least squares means for the measures for each day of observation are plotted in Figures 19 through 23.The results of correlation analyses for all variables are in Appendix D.

Least squares analysis of variance for percentage LFAT detected no TRT effect ($P=0.5057$) or a two-way interaction ($P=0.9827$); however, there was a trend for a DAY effect ($P\leq0.0803$; Table 31). Mean percentages of liver fat (LFAT) at d 21 prepartum, d +2, +14 and +28 postpartum for cows in the four TRT groups (I, II, III and IV) are in Table 34. Overall means for the percentage of LFAT across treatments on the four biopsy days (21 d prepartum, and d +2, +14 and +28 postpartum) were 6.70, 9.55, 11.57 and 9.47%, respectively (Table 33). For the last biopsy day (+28 DIM), there was a trend of a TRT effect among groups ($P\leq0.0970$). Percentages LFAT in livers of cows on TRT I, II, III and IV on d +28 were 8.45, 13.04, 8.04 and 8.33%, respectively (Table 34).

Neither orthogonal nor non-orthogonal contrasts of least squares means of LFAT percentages detected TRT effects. However, orthogonal contrasts of least squares means for DAY showed differences existed [contrast d -21 vs. d +2, +14 and +28 ($P\leq0.01$)] and the contrast d +14 vs. d +28 differed ($P\leq0.01$). Subsequent non-orthogonal contrast analysis of DAY effects indicated that percentage LFAT for d 21 prepartum differed from postpartum LFAT percentages (d +2, +14 and +28). Also, d +2 percentage of

LFAT differed from d +14 (P≤0.01), but not from d +28 (P=0.8887), and d +14 percentage LFAT differed from d +28 (P≤0.01; Table 33, Figure 19).

For percentage of TAG, results of least squares analysis of variance were similar to those results for percentage LFAT. In fact, LFAT and TAG percentages in liver were strongly and positively correlated (Appendix D). No TRT effect (P=0.4578) or two-way interaction (P=0.9793) were detected, but as for LFAT percentage, there was a trend for DAY effects (P≤0.0762) for percentage TAG (Table 31). Mean percentages of TAG at d 21 prepartum, and d +2, +14 and +28 postpartum for cows in TRT I, II, III and IV are in Table 34. Across all TRT groups, percentage TAG on d 21 d prepartum, and d +2, +14 and +28 postpartum were 1.15, 2.49, 3.51 and 2.65%, respectively (Table 33; Figure 20). Although no TRT effect was detected for percentages of LFAT across experimental groups for liver samples collected on d +28 postpartum, the TAG percentages of biopsy at d +28 did differ (P≤0.0486). On d +28, percentages of TAG for cows in the four TRT groups were 1.89, 4.49, 2.32 and 1.92%, respectively (Table 34).

Orthogonal and non-orthogonal comparisons of least squares means detected no TRT effects among experimental groups. However, for DAY the orthogonal contrast d -21 vs. d +2, +14 and +28 and orthogonal contrast d +14 vs. d +28 differed (P≤0.01). Subsequent non-orthogonal contrasts showed that percentage TAG on d 21 prepartum differed from that on d +2, +14 and +28 (P≤0.01), whereas d +2 postpartum TAG differed from d +14 (P≤0.01), but did not differ from d +28 (P=0.7225). Also d +14 TAG percentage differed from d +28 (P≤0.01; Table 33; Figure 20).

With regard to the mRNA abundance (gene expression) of the three liver enzymes evaluated, arbitrary units obtained from densitometry analysis were used in statistical analyses. Thus, all values of mRNA abundance for the liver enzymes are expressed as arbitrary units. For PC, no TRT (P=0.4737) or two-way interaction (P=0.3460) effects were observed, but there was a trend for DAY effect (P≤0.1020; Table 32). Mean PC mRNA expression at d 21 prepartum and for d +2, +14 and +28 postpartum for cows in TRT I, II, III and IV are in Table 35. Across all TRT groups, PC mRNA expression on d 21 prepartum and d +2, +14 and +28 postpartum were 172.6, 175.0, 173.6 and 171.0 arbitrary units, respectively (Table 33). For liver biopsies taken on d +28, there was a significant treatment (P≤0.0137) effect. The mean mRNA gene expression of PC in liver of cows on the four TRT groups on d +28 were 176.9, 169.7, 168.7 and 168.9 arbitrary units, respectively (Table 35).

Orthogonal and non-orthogonal contrasts of least squares means for PC did not detect any differences among TRT groups, but significant effects were seen for contrasts conducted to detect differences among least squares means on specific biopsy days (contrasts D, E and F). The contrast d +2 vs. d +14 and + 28 differed (P≤0.0215) and there was a trend for contrast d +14 vs. d+28 (P≤0.0876; Table 33) to differ. Non-orthogonal contrast analyses of least squares means showed that PC mRNA expression on d -21 differed from that on d +2 (P≤0.0385), and that d+2 differed from d +28 (P≤0.0069), but did not differ from d +14 (P=0.1947; Table 33; Figure 21).

For PEPCK, significant TRT (P≤0.0022) and DAY (P≤0.0001) effects were detected, but not the two-way interaction (P=0.2744; Table 32). Mean PEPCK mRNA expression in liver at d 21 prepartum, d +2, +14 and +28 postpartum for cows in TRT groups of cows (I, II, III and IV) are in Table 35. Across all TRT groups, mean PEPCK mRNA expression in liver of cows on the four treatment days were: 160.2, 166.0, 169.7

and 162.1 arbitrary units, respectively (Table 33). In addition, on d +28 postpartum, there was a significant TRT effect. Mean PEPCK mRNA expression was 165.0, 167.2, 162.4 and 153.8 arbitrary units for TRT I, II, III, and IV, respectively (Table 35, Figure 22). With regard to orthogonal contrasts, least squares means for TRT IV vs. II and III ($P \leq 0.0306$), and TRT II vs. III ($P \leq 0.01$) differed. Non-orthogonal contrasts showed that means for TRT I tended to differ from TRT III ($P \leq 0.0808$), and that it also differed from TRT IV cows ($P \leq 0.0317$). In addition, mRNA expression of PEPCK for TRT II cows differed from TRT IV cows ($P \leq 0.0013$). Evaluation of day effects (DAY) showed the contrast d -21 vs. d +2, +14 and +28, and the contrast d +14 vs. +28 were significant ($P \leq 0.01$). Non-orthogonal contrasts showed differences existed between d -21 and d +2 ($P \leq 0.0026$) and also d +14 ($P \leq 0.0001$). In addition, significant differences between means were detected for d +2 and d +28 postpartum ($P \leq 0.0455$), as well as between d +14 and d +28 postpartum ($P \leq 0.0010$; Table 33, Figure 22).

For MTP, mRNA expression patterns in liver were similar to those observed for PEPCK. Significant TRT ($P \leq 0.045$) and DAY ($P \leq 0.001$) effects were detected, but not for the two-way interaction ($P = 0.2163$; Table 32). Mean MTP mRNA expression at d 21 prepartum, and d +2, +14 and +28 postpartum for the four TRT groups are in Table 36. The mRNA expression of MTP on d 21 prepartum, d +2, +14 and +28 postpartum across all TRT were 179.9, 180.9, 173.9 and 168.2 arbitrary units, respectively (Table 32).

Across experimental groups of cows effects of TRT were detected for the third (+14 d) and fourth (+28 d) biopsy taken during the postpartum period to measure MTP mRNA gene expression. On d +14 and +28 postpartum, MTP expression was 181.1, 178.8, 164.0 and 171.6, and 169.2, 177.3, 167.7 and 158.8 arbitrary units, for the four treatment groups, respectively (Table 36). The orthogonal contrasts of least squares means detected a trend for the contrast TRT IV vs. II and III to differ ($P \leq 0.0642$). Non-orthogonal contrasts detected differences between least squares means for TRT I and IV ($P \leq 0.0295$) and between II and IV ($P \leq 0.0199$). The MTP mRNA expression for TRT IV cows was significantly less than for TRT I and II throughout the experiment (Table 33). Orthogonal contrasts of least squares means of MTP mRNA expression to evaluate DAY effects detected differences for contrasts d -21 vs. d +2, +14 and +28 ($P \leq 0.0137$), d +2 vs. d +14 and +28 ($P \leq 0.0001$) and d +14 vs. +28 ($P \leq 0.0210$). Non-orthogonal contrasts detected differences between d -21 prepartum and d +14 ($P \leq 0.03$) and d +28 ($P \leq 0.0001$) postpartum, between d +2 and +14 ($P \leq 0.0075$) and +28 postpartum ($P \leq 0.0001$), and between d +14 and +28 postpartum ($P \leq 0.0210$; Table 33; Figure 23).

Calving Variables

The calving variables considered were concentrations of immunoglobulins in colostrum, calf birth weight and calving difficulty. Clearly, these measures were only for the time period around calving. The mathematical model used to determine means for these three measures were treatment (TRT), calving season (SEA), and the two-way interaction. For colostrum immunoglobulins, no TRT ($P=0.4053$) or interaction effects ($P=0.8980$) were detected, but a SEA effect was detected ($P \leq 0.0020$; Table 37). Around calving, cows on TRT I, II, II and IV had mean colostrum immunoglobulin concentrations of 105.3, 91.4, 98.1 and 104.3 mg/mL in colostrum. All would be considered as good quality colostrum. Orthogonal and non-orthogonal contrast analyses did not detect any differences among these TRT means (Table 38).

Table 31. Least squares analyses of variance for percentages of fat (LFAT) and triacylglycerol (TAG) in liver of Holstein cows (wet weight basis) during prepartum and postpartum periods.

		LFAT[1]				TAG[1]		
Source	Df[2]	MS[2]	F[2]	Pr > F[2]	Df[2]	MS[2]	F[2]	Pr > F[2]
bST[3]	3	43.80	0.79	0.5057	3	11.45	0.89	0.4578
Day[4]	3	135.17	2.45	0.0803	3	32.25	2.50	0.0762
bST*Day	9	13.99	0.25	0.9827	9	3.45	0.27	0.9793
Cow (bST)[5]	34	55.16	3.85	0.0001	34	489.02	8.30	0.0001
Error	92	14.33			92	2.62		

[1] 39 multiparous Holstein cows from 21 d prepartum to +28 DIM.

[2] df (degrees of freedom), MS (Mean square), F (F-Statistic) and Pr>F (Probability).

[3] Treatments: I (Control), II (bST Post), III (bST Pre) and IV (bST Pre and Post).

[4] Day of biopsy: 21 d prepartum, +2, +14 and +28 d postpartum.

[5] Error term for sources above it.

Table 32. Least squares analyses of variance for mRNA expression in liver of Holstein cows for *pyruvate carboxylase* (PC), *phosphoenolpyruvate carboxykinase* (PEPCK) and *microsomal triacylglycerol transfer protein-1* (MTP).

	PC[1]				PEPCK[1]				MTP[1]			
Source	Df[2]	MS[2]	F[2]	Pr > F[2]	Df[2]	MS[2]	F[2]	Pr > F[2]	Df[2]	MS[2]	F[2]	Pr > F[2]
bST[3]	3	23.40	0.87	0.4737	3	229.69	6.95	0.0022	3	309.59	3.21	0.0450
Day[4]	3	63.59	2.36	0.1020	3	393.72	11.91	0.0001	3	782.14	8.11	0.0010
bST*Day	9	32.43	1.20	0.3460	9	44.61	1.35	0.2744	9	144.17	1.50	0.2163
Cow (bST)[5]	20	31.00	1.24	0.2588	20	218.75	4.76	0.0001	20	344.76	4.21	0.0001
Ribo	1	253.92	10.17	0.0024	1	4909.24	106.73	0.0001	1	3344.20	40.88	0.0001
Error	54	24.96			54	45.99			54	81.80		

[1] 39 multiparous Holstein cows from 21 d prepartum to +28 DIM.

[2] df (degrees of freedom), MS (Mean square), F (F-Statistic) and Pr>F (Probability).

[3] Treatments: I (Control), II (bST Post), III (bST Pre) and IV (bST Pre/Post).

[4] Day of biopsy: 21 d prepartum, +2, +14 and +28 d postpartum.

[5] Error term for sources above it.

Table 33. Least squares means and SEM for percentages of fat (LFAT) and triacylglycerol (TAG) in liver of Holstein cows on a wet weight basis, and for liver mRNA expression of *pyruvate carboxylase* (PC), *phosphoenolpyruvate carboxykinase* (PEPCK) and *microsomal triacylglycerol transfer protein-1* (MTP) for treatments and day from 21 d prepartum to +28 DIM[1].

	Treatments					Orthogonal Comparisons			
	Control (n=8)	bST Post (n=10)	bST Pre (n=10)	bST Pre/Post (n=11)	SEM	Overall	A[2]	B[3]	C[4]
Variables						----------*P*----------			
LFAT, %	8.66	10.94	8.51	9.18	1.29	NS	NS	NS	NS
TAG, %	1.82	3.20	2.59	2.18	0.61	NS	NS	NS	NS
PC, units	174.65	172.70	172.38	172.75	1.10	NS	NS	NS	NS
PEPCK, units	165.98^{ab}	168.28^{b}	162.70^{ad}	161.21^{cd}	1.22	**	NS	**	**
MTP, units	178.77^{a}	179.03^{a}	173.79^{ab}	171.50^{b}	2.09	**	NS	†	NS
	Day								
	-21	+2	+14	+28	SEM	Overall	D[5]	E[6]	F[7]
LFAT, %	6.70^{a}	9.55^{b}	11.57^{c}	9.47^{bd}	1.27	†	**	NS	**
TAG, %	1.15^{a}	2.49^{b}	3.51^{c}	2.65^{bd}	0.61	†	**	†	**
PC, units	172.63^{ac}	175.07^{b}	173.68^{abd}	171.08^{cd}	1.11	†	NS	*	†
PEPCK, units	160.22^{a}	166.08^{b}	169.75^{b}	162.12^{a}	1.23	**	**	NS	**
MTP, units	179.92^{a}	180.99^{a}	173.92^{b}	168.26^{bc}	2.10	**	**	**	*

[1] 39 multiparous Holstein cows. [2] Contrast A: I (Control) vs. II (bST Post) + III (bST Pre) + IV (bST Pre/Post). [3] Contrast B: IV (bST Pre/Post) vs II (bST Post) + III (bST Pre). [4] Contrast C: II (bST Post) vs. III (bST Pre/Post). [5] Contrast D: d 21 vs. d +2, +14 and +28. [6] Contrast E: d +2 vs. +14 and +28. [7] Contrast F: d +14 vs. +28. [a,b,c,d] Superscripts that differ in rows indicate significance ($P \leq 0.05$) for non-orthogonal contrasts. † $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$.

Table 34. Least squares means and SEM for percentages of fat (LFAT) and triacylglycerol (TAG) in liver of Holstein cows on a wet weight basis for each day of biopsy[1].

	LFAT					Orthogonal Comparisons			
	Control (n=8)	bST Post (n=10)	bST Pre (n=10)	bST Pre/Post (n=11)	SEM	Overall	A[3]	B[4]	C[5]
Day[2]						-----	----- P -----	-----	-----
-21	6.81	7.30	6.43	6.25	2.51	NS			
+2	9.20	9.30	9.50	10.20	2.49	NS			
+14	10.16	14.10	10.08	11.95	2.64	NS			
+28	8.45^{a}	13.04^{b}	8.04^{a}	8.33^{a}	2.51	†			
	TAG								
	Control (n=8)	bST Post (n=10)	bST Pre (n=10)	bST Pre/Post (n=11)	SEM	Overall	A[3]	B[4]	C[5]
- 21	0.08	1.30	1.59	0.90	1.21	NS			
+2	2.02	2.46	3.14	2.33	1.20	NS			
+14	2.58	4.56	3.32	3.57	1.27	NS			
+28	1.89^{a}	4.49^{b}	2.32^{a}	1.92^{a}	1.21	*			

[1] 39 multiparous Holstein cows from 21 d prepartum to +28 DIM. [2] Days from calving. [3] Contrast A: I (Control) vs. II (bST Post) + III (bST Pre) + IV (bST Pre/Post). [4] Contrast B: IV (bST Pre/Post) vs II (bST Post) + III (bST Pre). [5] Contrast C: II (bST Post) vs. III (bST Pre/Post). [a,b,c] Superscripts that differ in rows indicate significance ($P \leq 0.05$) for non-orthogonal contrasts. † $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$.

Table 35. Least squares means and SEM for mRNA expression in liver of Holstein cows for *pyruvate carboxylase* (PC) and *phosphoenolpyruvate carboxykinase* (PEPCK) for each day of biopsy [1].

	PC					Orthogonal Comparisons			
	Control (n=8)	bST Post (n=10)	bST Pre (n=10)	bST Pre/Post (n=11)	SEM	Overall	A[3]	B[4]	C[5]
Day[2]						P			
-21	174.37	170.72	171.12	174.33	2.26	NS			
+2	174.75	176.01	176.38	173.16	2.25	NS			
+14	172.56	174.34	173.22	174.60	2.21	NS			
+28	176.91^{a}	169.73^{b}	168.78^{b}	168.90^{b}	2.15	**			
	PEPCK								
	Control (n=8)	bST Post (n=10)	bST Pre (n=10)	bST Pre/Post (n=11)	SEM	Overall	A[3]	B[4]	C[5]
-21	159.01^{a}	164.74^{a}	157.30^{b}	159.84^{ab}	2.50	NS			
+2	169.45	169.57	163.64	161.66	2.49	NS			
+14	170.44	171.59	167.46	169.51	2.45	NS			
+28	165.02^{a}	167.23^{a}	162.40^{a}	153.84^{b}	2.38	**			

[1] 39 multiparous Holstein cows from 21 d prepartum to +28 DIM. [2] Days from calving. [3] Contrast A: I (Control) vs. II (bST Post) + III (bST Pre) + IV (bST Pre/Post). [4] Contrast B: IV (bST Pre/Post) vs II (bST Post) + III (bST Pre). [5] Contrast C: II (bST Post) vs. III (bST Pre/Post). [a,b,c] Superscripts that differ in rows indicate significance ($P \leq 0.05$) for non-orthogonal contrasts. $^{+} P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$.

Table 36. Least squares means and SEM for mRNA expression in liver of Holstein cows for *microsomal triacylglycerol transfer protein 1* (MTP) for each day of biopsy [1].

	MTP					Orthogonal Comparisons			
	Control (n=8)	bST Post (n=10)	bST Pre (n=10)	bST Pre/Post (n=11)	SEM	Overall	A[3]	B[4]	C[5]
Day[2]						-----------------------------	-------P-------	-----------------------------	
-21	180.05	179.85	179.89	179.87	4.27	NS			
+2	184.70	180.13	183.50	175.63	4.26	NS			
+14	181.13^{ac}	178.84^{ac}	164.07^{b}	171.65^{bc}	4.19	**			
+28	169.20^{ac}	177.30^{a}	167.72^{ac}	158.83^{bc}	4.07	**			

[1] 39 multiparous Holstein cows from 21 d prepartum to +28 DIM. [2] Days from calving. [3] Contrast A: I (Control) vs. II (bST Post) + III (bST Pre) + IV (bST Pre/Post). [4] Contrast B: IV (bST Pre/Post) vs II (bST Post) + III (bST Pre). [5] Contrast C: II (bST Post) vs. III (bST Pre/Post). [a,b,c] Superscripts that differ in rows indicate significance ($P \leq 0.05$) for non-orthogonal contrasts. $^{\dagger} P \leq 0.10$; $^{*} P \leq 0.05$; $^{**} P \leq 0.01$.

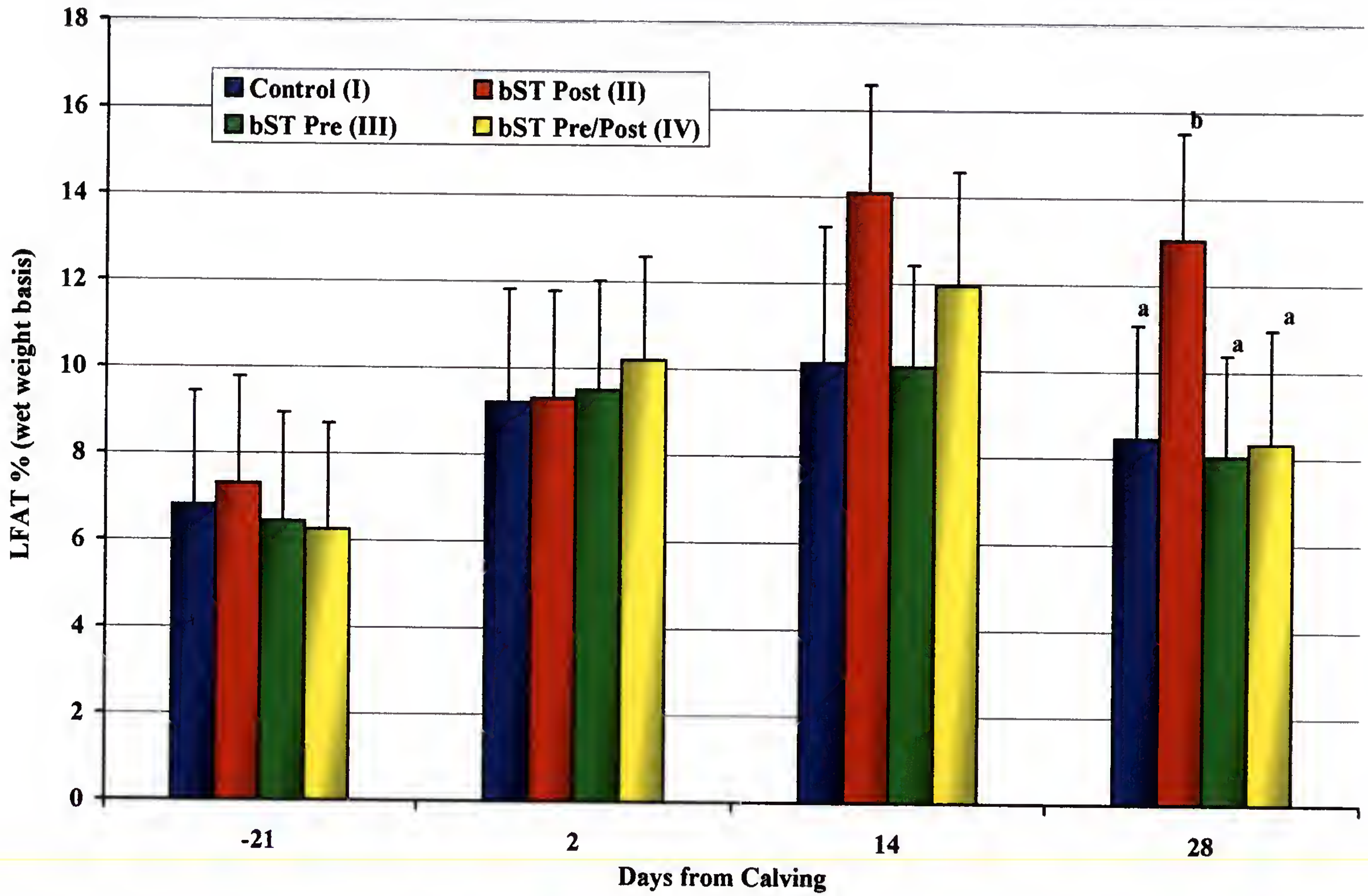

Figure 19. Least squares means depicting percentages of total fat in liver (LFAT, wet weight basis) of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. ^{a,b} Superscripts within day from calving indicate means that differed at $P<0.05$.

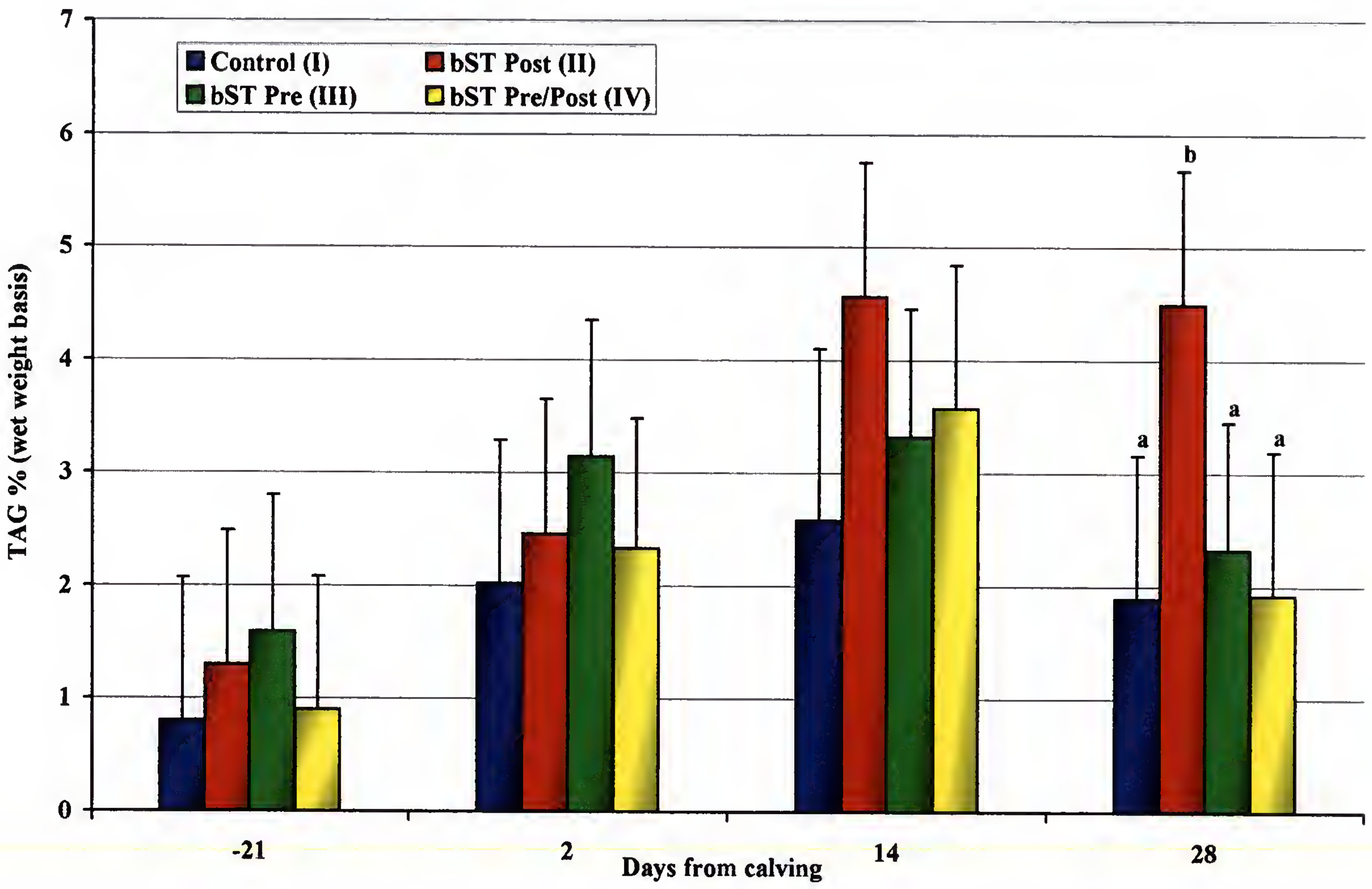

Figure 20. Least squares means depicting percentage of triacylglycerol in liver (TAG, wet weight basis) of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. ^{a,b} Superscripts within day from calving indicate means that differed at $P<0.05$.

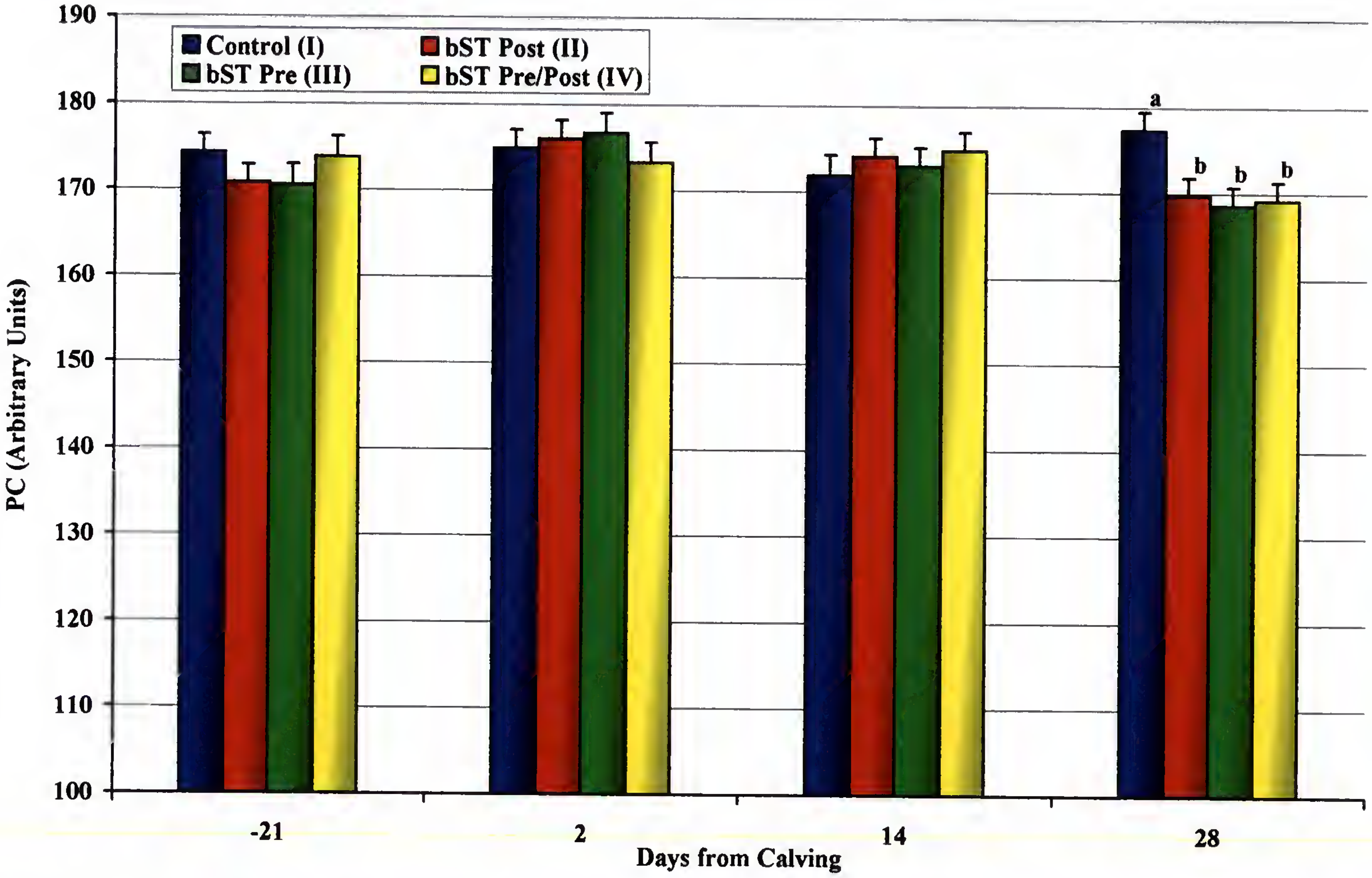

Figure 21. Least squares means depicting mRNA expression of *pyruvate carboxylase* (PC) of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. ^{a,b} Supercripts within day from calving indicate means that differed at P<0.05.

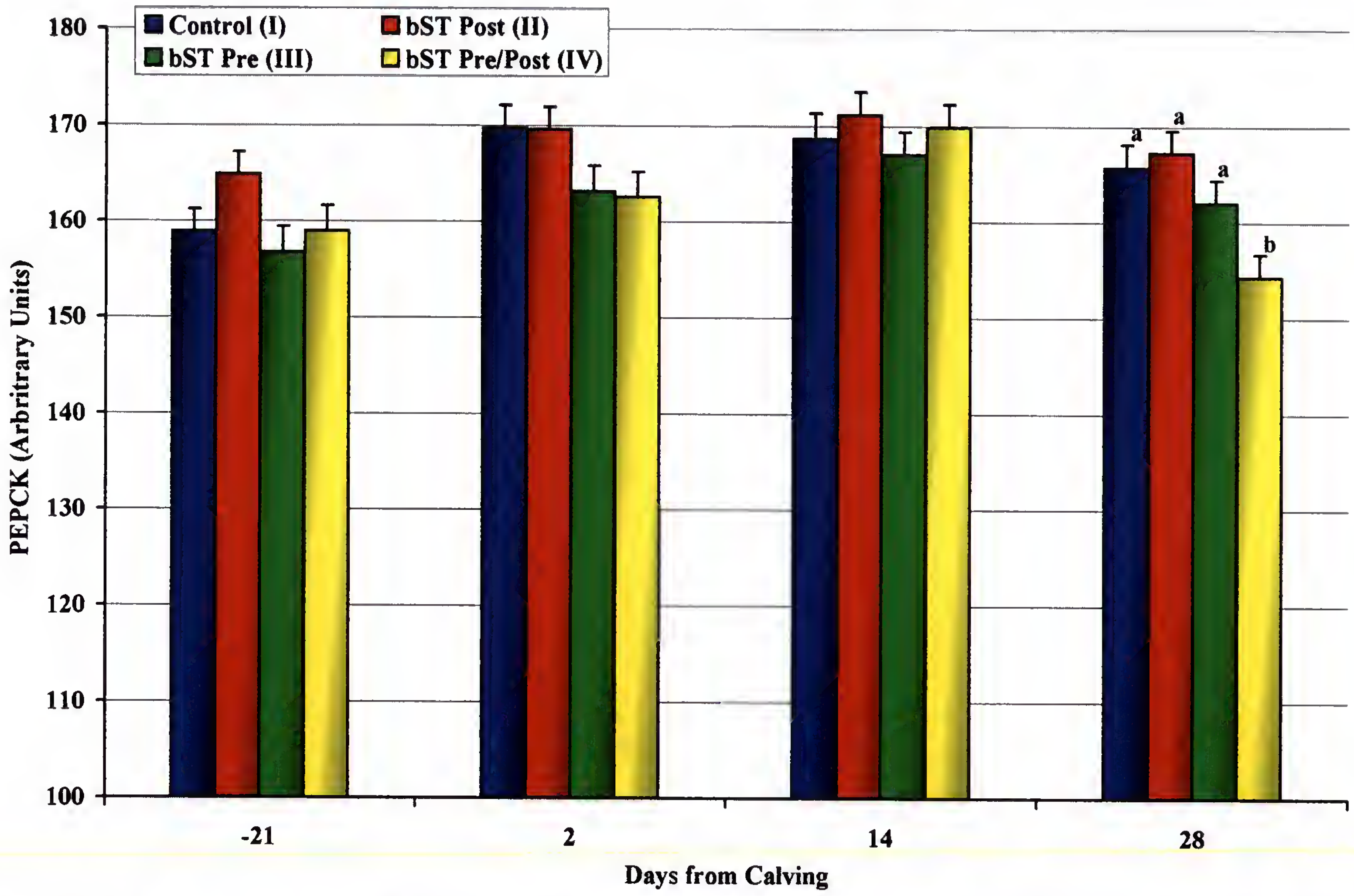

Figure 22. Least squares means depicting mRNA expression of *phosphoenolpyruvate carboxykinase* (PEPCK) of multiparous Holstein cows supplemented or not with bST during the prepartum and/or periods. ^{a,b} Superscripts within day from calving indicate means that differed at P<0.05.

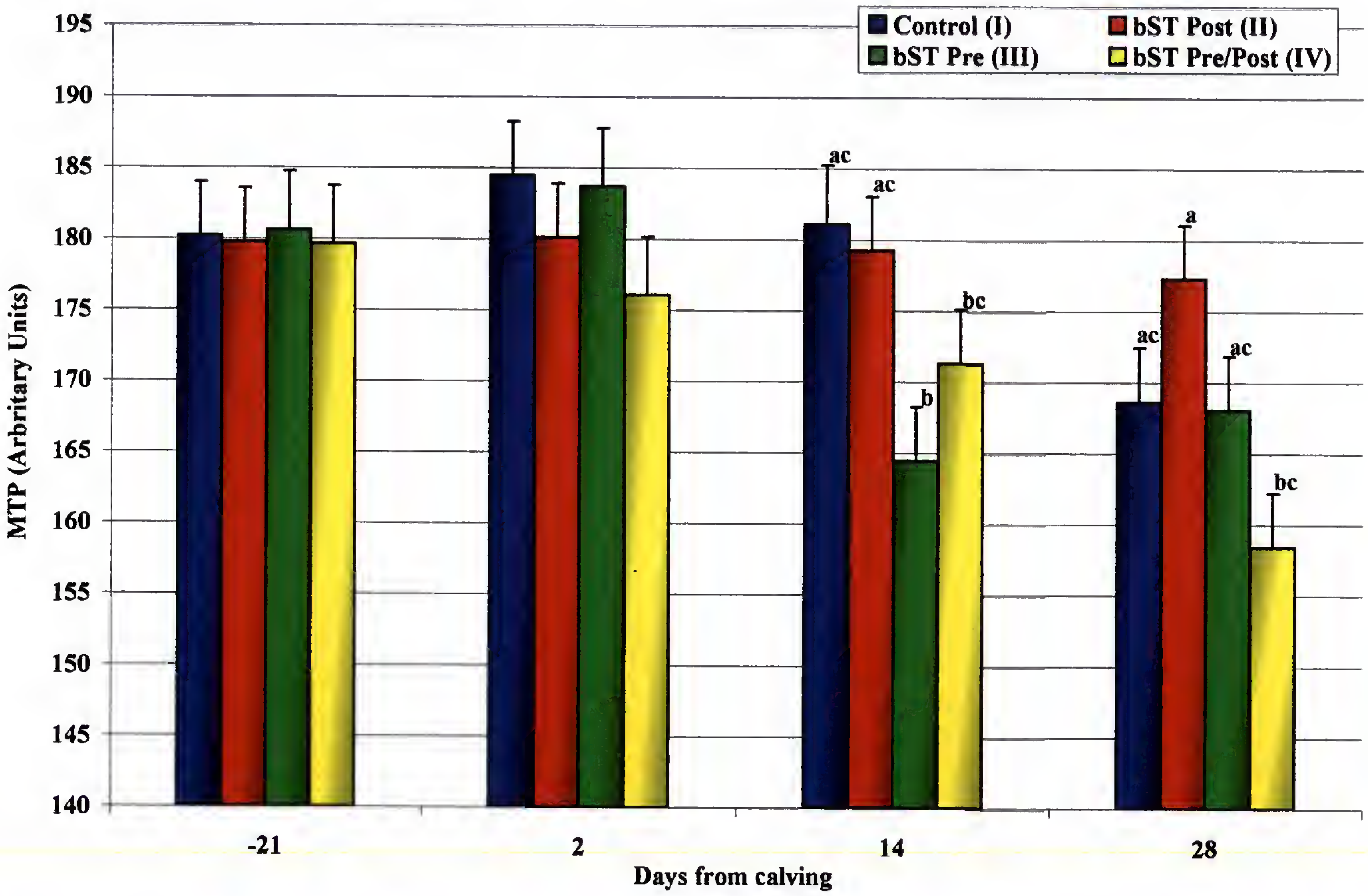

Figure 23. Least squares means depicting mRNA expression of *microsomal triacylglycerol transfer protein 1* (MTP) of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. ^{a,b} Superscripts within day from calving indicate means that differed at $P<0.05$.

For calf birth weight, no TRT (P=0.6600) or SEA (P=0.1501) effects were detected, but an interaction trend was seen (P=0.0907; Table 37). The mean calf birth weights for cows on the four treatments were 40.3, 37.9, 39.0 and 39.1 kg, respectively (Table 38). As for colostrum, orthogonal and non-orthogonal contrast analyses did not detect any differences among the TRT means. With regard to difficulty of calving, a significant TRT (P≤0.0001) effect was detected, but no SEA effect (P=0.1402) or interaction (P=0.6679; Table 37). The calving difficulty scores of cows on the four treatments were 2.04, 1.19, 1.23 and 1.03 pt., respectively (Table 38). Orthogonal contrasts detected significant TRT effects for contrast of TRT I, vs. II, III and IV (P≤0.0071), and the non-orthogonal contrasts showed that TRT I means differed from TRT II, III and IV (P<0.0183; Table 38).

Health Information and Reproductive Performance

Data collected to evaluate health and used in the statistical analyses were disease incidences (number of new cases of a disease in a TRT group during the first 60 DIM) of retained fetal membranes (RFM), metritis (MET), mastitis (MAST), digestive problems (DIG), ketosis (KETO), milk fever (MF), left/right displaced abomasum (L/R DA), lameness (LAME), and total number of sick animals in a group (SICK). For reproductive performance, the variables considered were conception rate at first service (CRFS), pregnancy rates (PR), services per conception (SPC), and the calving to conception interval (CCI).

For health data, statistical analyses evaluated differences between control group (TRT I) and other TRT groups, plus the cohorts, and also between calving seasons (SEA), regardless of TRT. For two measures of reproductive performance (variables

Table 37. Least squares analyses of variance for colostrum immunoglobulin content, calf birth weight and calving difficulty of Holstein cows[1].

	Colostrum				Calf Birth Weight				Calving Difficulty			
Source	Df[2]	MS[2]	F[2]	Pr > F[2]	Df[2]	MS[2]	F[2]	Pr > F[2]	Df[2]	MS[2]	F[2]	Pr > F[2]
bST[3]	3	884.98	4.90	0.4053	4	187.13	0.29	0.6600	4	4.04	11.28	0.0001
Season[4]	1	9191.28	51.00	0.0020	1	648.30	1.01	0.1501	1	1.33	1.66	0.1402
bST*Season[5]	3	177.69	0.20	0.8980	4	634.26	2.05	0.0907	4	0.36	0.59	0.6679
Error	83	901.16			141	309.53				0.60		

[1] 103 multiparous Holstein cows.

[2] df (degrees of freedom), MS (Mean square), F (F-Statistic) and Pr>F (Probability).

[3] Treatments: I (Control), II (bST Post), III (bST Pre) and IV (bST Pre/Post).

[4] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb).

[5] Error term for sources above it.

Table 38. Least squares means and SEM for colostrum immunoglobulin content (Colost), calf birth weight (CLFWT) and calving difficulty (DCV) of Holstein cows for treatment groups and season of calving.

	Treatments					Comparisons			
	Control (n=26)	bST Post (n=25)	bST Pre (n=27)	bST Pre/Post (n=25)	SEM	Overall	A[2]	B[3]	C[4]
Variable						------------------------------	---------------P	----------------------------	
Colost, mg/mL[1]	105.3	91.4	98.1	104.3	2.82	NS	NS	NS	NS
CLFWT, kg[1]	40.36	37.90	39.03	39.15	2.24	NS	NS	NS	NS
DCV, pt.[1,6]	2.04^{a}	1.19^{b}	1.23^{b}	1.03^{b}	0.11	**	**	NS	NS
		Season[5]							
		I (n=52)	II (n=51)		SEM	Overall			
Colost, mg/mL[1]		109.9	89.6		1.99	**			
CLFWT, kg[1]		37.8	39.7		1.35	NS			
DCV, pt.[1]		1.25	1.44		0.07	NS			

[1] 103 multiparous Holstein cows. [2] Contrast A: I (Control) vs. II (bST Post) + III (bST Pre) + IV (bST Pre/Post). [3] Contrast B: IV (bST Pre/Post) vs II (bST Post) + III (bST Pre). [4] Contrast C: II (bST Post) vs. III (bST Pre/Post). [5] Cows were grouped into two calving seasons, I (Aug-Dec) and II (Jan-April). [a,b] Superscripts that differ indicate significance ($P \leq 0.05$) for non-orthogonal contrasts. [6] Assistance at calving required [1 (no assistance) – 5 (c-section required)]. † $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$.

CRFS and PR), statistical methods were used to determine differences between control group (TRT I) and the other TRT groups and cohorts, whereas for the two variables, SPC and CCI, the analyses determined least squares means for these measures among all treatment (TRT) groups and during the two calving seasons (SEA).

From the initial statistical analysis of health data, the diseases that showed greatest incidence (>10%) across all TRT groups were RFM, MET, MAST and DIG (Table 39). The percentages of SICK cows in each of the four treatment groups plus cohorts during the first 60 DIM were 76.9, 64.0, 59.2, 48.0 and 65.9%, respectively (Table 39). Using the test of Heterogeneity of Proportions (Khouri, 2000), it was observed that cows in TRT group IV were 7.4-times less likely to have a RFM or DIG case than controls ($P \leq 0.05$). In addition, there was a tendency for cows in TRT group IV to be one-fifth as likely to become sick compared to control cows ($P \leq 0.10$). With regard to SEA, the only significant association was for DIG. Across all TRT groups, cows that calved during SEA II (Nov-Feb) were 5.7-times more likely to develop a DIG problem compared to cows that calved during SEA I (Aug-Oct; $P \leq 0.01$). No other associations between any TRT group and controls were significant ($P > 0.10$). In addition to the described effects between TRT group IV cows and controls, across all TRT groups of cows, several other associations between diseases were seen. Cows with RFM were 4.7-times more likely to develop MET ($P \leq 0.05$), and cows with ketosis tended to be 6.2-times more likely to develop a DIG problem ($P \leq 0.10$; Table 40).

With regard to reproductive performance variables, the CRFS of cows in the four TRT groups and cohorts were 37.5, 36.3, 26.3, 47 and 25%, respectively (Table 39). The PR of cows in TRT groups I, II, III, IV and cohorts were 57.6, 88, 70.3, 68, and 51%, respectively (Table 39). In addition, there was a tendency for an association between TRT I and TRT II groups of cows for PR. Cows on TRT II tended to be 4.5-times more likely to become pregnant during the subsequent lactation than control cows ($P \leq 0.10$; Table 40). For CCI no TRT (P=0.8938) or SEA (P=0.6578) effects were detected. Similarly, no TRT (P=0.9590) or SEA (P=0.5624) effects were seen for SPC. The CCI and SPC of cows on TRT I, II, III, IV and for the cohorts were 136.0 and 2.68; 170.6 and 3.10; 157.0 and 2.93; 159.9 and 3.58; 174.7 and 3.13, respectively.

Milk Yield and Milk Composition

In addition to evaluating metabolic adaptations, liver metabolism and health of dairy cows supplemented or not supplemented with low doses of bST during the prepartum and early lactation periods, an important and major objective of this experiment was to observe effects upon milk production and milk composition during the subsequent lactation. Therefore, data for daily milk yields (MY) collected from 3-150 DIM and the milk produced during the actual 305-d lactation obtained from DHIA records were used in the statistical analyses. The daily milk production was analyzed over three time periods, from 3-63 DIM, 64-150 DIM and from 3-150 DIM. The 3-150 DIM lactation period was divided into two phases, because beginning at 64 DIM, all cows, including non-supplemented controls, were supplemented with the full dose of bST (500 mg/14d of POSILAC®, Monsanto Company St. Louis, MO); the dose typically used to enhance lactation milk yield. The percentages of milk fat (FAT), protein (PROT) and somatic cell counts (SCC) in milk also were evaluated. These measures were collected biweekly from 3-63 DIM and also were subjected to statistical analyses. All data were analyzed using the first and second statistical models to obtain and contrast least squares

Table 39. Disease incidence and reproductive performance of multiparous Holstein cows supplemented or not with bST[1].

	Control (I, n=26)	bST Post (II, n=25)	bST Pre (III, n=27)	bST Pre & Post (IV, n=25)	Cohorts (n=47)
			Incidence[2]		
Disease[3]					
RFM, n[4] (%)[5]	5 (19.2)	4 (16.0)	2 (7.4)	0 (0.0)	12 (25.5)
MET, n (%)	11 (42.3)	8 (32.0)	10 (37.0)	8 (32.0)	20 (42.5)
MAST, n (%)	8 (30.7)	7 (28.0)	4 (14.8)	5 (20.0)	10 (21.2)
DIG, n (%)	5 (19.2)	4 (16.0)	3 (11.1)	0 (0.0)	4 (8.5)
KETO, n (%)	1 (3.8)	2 (8.0)	2 (7.4)	0 (0.0)	1 (2.1)
MF, n (%)	2 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
L/R DA, n (%)	0 (0.0)	2 (8.0)	1 (3.7)	0 (0.0)	2 (4.2)
LAME, n (%)	1 (3.8)	1 (4.0)	1 (3.7)	1 (4.0)	3 (6.3)
SICK, n (%)	20 (76.9)	16 (64.0)	16 (59.2)	12 (48.0)	31 (65.9)
Reproductive Variable[6]					
CRFS, n (%)	6 (37.5)	8 (36.3)	5 (26.3)	8 (47.0)	6 (25.0)
PR, n (%)	15 (57.6)	22 (88.0)	19 (70.3)	17 (68.0)	24 (51.0)
CCI, d	136.0	170.6	157.0	159.9	174.7
SPC	2.68	3.10	2.93	3.58	3.13

[1] 103 cows milked 3x/d. [2] Number of cows with a new case of a disease during the first 60 DIM. [3] Retained fetal membranes (RFM), metritis (MET), mastitis (MAST), digestive problems (DIG), ketosis (KETO), milk fever (MF), left/right displaced abomasum (L/R DA), lameness (LAME) and total number of sick animals in a group (SICK). [4] Number of cases. [5] Percentage of cows showing cases in a group. [6] Conception rate at first service (CRFS), pregnancy rates (PR), calving to conception interval (CCI) and services per conception (SPC).

Table 40. Summary of logistic regression analyses of the associations between TRT, SEA and diseases, and variables of reproductive performance of multiparous Holstein cows during 0-60 DIM[1].

Model		Coefficients		
Dependent[2]	Independent	OR[3]	95% CI[4]	P-value
RFM	bST Post (II)	0.8	0.18-3.40	NS
	bST Pre (III)	0.3	0.05-1.91	NS
	bST Pre/Post (IV)[3]	7.4	1.88-165.4	≤0.05
	Dystocia[5]	0.9	0.26-3.62	NS
MET	bST Post (II)	0.6	0.20-2.01	NS
	bST Pre (III)	0.8	0.26-2.41	NS
	bST Pre/Post (IV)	0.6	0.20-2.01	NS
	RFM[6]	4.7	1.83-12.55	≤0.01
MAST	bST Post (II)	0.8	0.26-2.92	NS
	bST Pre (III)	0.3	0.10-1.50	NS
	bST Pre/Post (IV)	0.5	0.15-2.03	NS
DIG	bST Post (II)	0.8	0.18-3.40	NS
	bST Pre (III)	0.5	0.11-2.46	NS
	bST Pre/Post (IV)[3]	7.4	1.88-165.4	≤0.05
	Ketosis	6.2	1.00-39.06	≤0.07
	SEA[7]	5.7	1.59-20.81	≤0.01
SICK	bST Post (II)	0.5	0.15-1.81	NS
	bST Pre (III)	0.4	0.13-1.43	NS
	bST Pre/Post (IV)	0.2	0.08-0.92	≤0.10
CRFS	bST Post (II)	1.5	0.45-5.42	NS
	bST Pre (III)	0.7	0.20-2.87	NS
	bST Pre/Post (IV)	1.5	0.45-5.42	NS
PR	bST Post (II)[8]	4.5	1.08-19.38	≤0.10
	bST Pre (III)	1.4	0.47-4.65	NS
	bST Pre/Post (IV)	1.3	0.41-4.20	NS

[1] 103 cows milked 3x/d. [2] Retained fetal membranes (RFM), metritis (MET), mastitis (MAST), digestive problems (DIG), total number of sick animals in a group (SICK), conception rate at first service (CRFS) and pregnancy rates (PR). [3] Odds ratio referent to control group. Cows exposed to TRT IV (bST Pre/Post) were 7.4 times less likely to develop RFM and DIG. [4] 95% confidence interval for odds ratio. [5] Cows that required assistance during calving (Assistance to calving required [1 (no assistance) – 5 (c-section required)]. [6] Cows exposed to RFM were 4.7 times more likely to have MET. [7] Cows that calved during season II (Nov-Feb) were 5.7 times more likely to have digestive problems than cows that calved during season I (Aug-Oct). [8] Cows exposed to TRT II tended to be more pregnant at the end of the subsequent lactation.

means, to describe overall trends during the postpartum period, to compare results across treatment (TRT) groups and calving seasons (SEA), and to determine if there was an interaction between TRT and SEA.

From least squares analysis of variance for MY during 0-63 DIM, no overall TRT (P=0.1252), SEA (P=0.5131) or two-way interaction (P=0.5269) effects were detected (Table 41). However, by orthogonal contrast analysis of TRT least squares means, a trend of a TRT effect was detected (TRT I vs. TRT II, III and IV; P≤0.0674). Subsequent non-orthogonal contrasts showed that the only difference was between means for MY of cows on TRT IV vs. TRT I during this time period (P≤0.019). The regression curves depicted in Figure 25 illustrate this effect. No other orthogonal or non-orthogonal comparisons of the least squares means were significant during this period of early lactation. The mean MY of cows on TRT I, II, III and IV were 34.81, 37.05, 38.32 and 41.32 kg/d, respectively (Table 43).

During 64-150 DIM, the time during which all cows were supplemented with the full-dose of bST, one cow was removed from trial due to severe mastitis and lameness, therefore data from only 102 cows were used in the statistical analyses. From least squares analysis of variance, no TRT (P=0.6562), SEA (P=0.5783) or two-way interaction (P=0.7297) effects were detected among TRT groups (Table 41). In addition, no significant differences were detected among least squares means of daily milk production by orthogonal or non-orthogonal contrasts. The results for milk production are depicted in Figures 24 and 25. It is clear that MY of the four groups of cows tended to come together over this 12+ week time period, compared to more distinct separation during the first 63 DIM. During 64-150 DIM, cows on TRT I, II, III and IV had mean MY of 36.06, 36.89, 38.99 and 39.24 kg/d, respectively (Table 43).

Statistical analysis of data collected up to 150 DIM detected no TRT (P=0.2962), SEA (P=0.5749) or two-way interaction (P=0.7992) effects (Table 42). No differences in TRT least squares means were detected by orthogonal contrasts. From 3-150 DIM, cows in TRT groups I, II, III and IV produced mean daily MY of 36.12, 37.68, 38.01 and 40.76 kg/d (Table 43). Using non-orthogonal contrast analyses, a significant difference between mean milk yield (kg/d) for contrast of TRT I vs. IV cows (36.12 vs. 40.76 kg/d; +12.8%; $P \leq 0.0565$) was seen.

To evaluate total milk production during the actual 305-d lactation, data from a total of 89 cows that completed the full 305-d lactation were analyzed using the previous 305-d lactation MY as a covariate. Numbers of cows per TRT group that completed the 305-d lactation were 23, 22, 25 and 19, respectively. From the least squares analysis of variance, no TRT (P=0.2181) or SEA (P=0.4148) effects were detected among TRT groups; similar results were seen using orthogonal contrasts of least square means (3-150 DIM). However, non-orthogonal contrasts detected a trend for the contrast TRT I vs. IV ($P \leq 0.0752$). Mean total milk production during the actual 305-d lactation period for cows on TRT I, II, III and IV were 9,920; 10,500; 10,702 and 11,884 kg of milk, respectively. This calculated out to be mean daily milk yields of 32.52, 34.42, 35.08 and 38.96 kg/d. Interestingly, although not significant, mean total and daily (kg/d) MY in the three bST-supplemented groups were greater than that of the non-supplemented control group.

Because a TRT effect between TRT I and IV was detected during the first half of the lactation (3-150 DIM), regression analyses were performed for MY. Tests of heterogeneity of regression were completed using the general model to partition the sources of variation for treatments into single degrees of freedom. The MY variable was evaluated with calving season (SEA), cow(SEA) and the covariate day to the third order polynomial; again using cow(season) as error the term. The overall cubic regression was significant (curvilinear, $P \leq 0.0001$).

Regression coefficients and the intercepts for MY for each of the four treatments are in Table 44 and the regression curves are plotted in Figure 25. These polynomial curves demonstrate that milk production in all bST supplemented groups of cows were greater at the beginning of the lactation than for non-supplemented controls, and especially that there were clear differences between TRT I and IV during the first 63 DIM. Following supplementation of the full dose of bST beginning at 64 DIM (500 mg/14d), levels of milk production in groups III and IV still were greater than controls throughout the period, but the decline in production was faster as lactation progressed, or conversely, less rapid in the control group of cows (Figure 26). Heterogeneity of regression of the four regression curves indicated that they were not parallel (Appendix C).

Least squares analyses of variance for percentages milk components detected no TRT effects for any of the three measures [FAT ($P=0.7452$), PROT ($P=0.6019$) or SCC ($P=0.3387$)]. However, significant effects of calving season (SEA) were detected for percentages of fat ($P \leq 0.0354$) and protein ($P \leq .0001$) in milk, but not for SCC ($P=0.1433$). No two-way interactions were detected for FAT ($P=0.9303$), PROT ($P=0.7514$) or SCC ($P=0.1670$; Table 45). Mean percentages of fat and protein, and SCC for cows on TRT I, II, III and IV were 3.82, 2.90 and 512.92; 3.78, 2.83 and 608.51; 3.85, 2.80 and 312.35;

and 3.72%, 2.80% and 522.33 x 10^3 cells/mL, respectively (Table 46). Similarly, no significant differences among TRT least squares means were detected by either orthogonal or non-orthogonal contrasts, except for the trend of a difference detected between TRT II vs. III for SCC ($P \leq 0.0817$).

Table 41. Least squares analyses of variance for milk yield of Holstein cows during 3-63 and 64-150 DIM.

	3-63 DIM[1]				64-150 DIM[2]			
Source	Df[3]	MS[3]	F[3]	Pr > F[3]	Df[3]	MS[3]	F[3]	Pr > F[3]
bST[4]	3	10772.71	1.96	0.1252	3	3623.74	0.54	0.6562
Season[5]	1	2368.27	0.43	0.5131	1	2088.90	0.31	0.5783
bST*Season	3	4103.97	0.75	0.5269	3	2908.46	0.43	0.7297
Cow (bST*Season)[6]	95	5493.34	119.00	0.0001	94	6678.83	190.92	0.0001
Day	1	36586.08	792.55	0.0001	1	12121.27	346.49	0.0001
Day*Day	1	26535.83	574.84	0.0001	1	738.28	21.10	0.0001
Day*Day*Day	1	3166.41	68.59	0.0001	1	239.98	6.86	0.0088
Error	5904	46.16			9056	34.98		

[1] 103 multiparous Holstein cows milked 3x/day.

[2] 102 multiparous Holstein cows milked 3x/day.

[3] df (degrees of freedom), MS (Mean square), F (F-Statistic) and Pr>F (Probability).

[4] Treatments: I (Control), II (bST Post), III (bST Pre) and IV (bST Pre/Post).

[5] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb).

[6] Error term for sources above it.

Table 42. Least squares analyses of variance for milk yield of Holstein cows during 3-150 DIM and for Actual 305-d MY.

	3-150 DIM[1]				Actual 305-d MY[2]			
Source	Df[3]	MS[3]	F[3]	Pr > F[3]	Df[3]	MS[3]	F[3]	Pr > F[3]
bST[4]	3	13485.19	1.25	0.2962	3	13054420.3	2.70	0.2181
Season[5]	1	3417.44	0.32	0.5749	1	4308413.3	0.89	0.4148
bST*Season	3	3627.60	0.34	0.7992	3	2294033.3	0.50	0.6858
Cow (bST*Season)[6]	95	10814.9	218.94	0.0001	--			
Day or Prev. MY (305-d)[7]	1	1854.4	37.54	0.0001	1	53184541.0	11.51	0.0011
Day*Day	1	46750.3	946.40	0.0001	--			
Day*Day*Day	1	12125.9	245.47	0.0001	--			
Error	15170	49.39			88	4621479.7		

[1] 103 multiparous Holstein cows milked 3x/day.

[2] 89 multiparous Holstein cows milked 3x/day.

[3] df (degrees of freedom), MS (Mean square), F (F-Statistic) and Pr>F (Probability).

[4] Treatments: I (Control), II (bST Post), III (bST Pre) and IV (bST Pre and Post).

[5] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb).

[6] Error term for sources above it for 3-150 DIM.

[7] Previous 305-d lactation MY used as covariate for Actual 305-d MY.

Table 43. Least squares means and SEM for milk yield (MY) of Holstein cows for treatments and season of calving.

	Treatments					Comparisons			
	Control (n=26)	bST Post (n=25)	bST Pre (n=27)	bST Pre/Post (n=25)	SEM	Overall	A[5]	B[6]	C[7]
Variable						------------	------------ *P*	------------	------------
MY, kg/d[1]	34.81^{a}	37.05^{ab}	38.32^{ab}	41.32^{b}	1.91	NS	†	NS	NS
MY, kg/d[2]	36.06	36.89	38.99	39.24	2.23	NS	NS	NS	NS
MY, kg/d[3]	36.12^{a}	37.68^{ab}	38.01^{ab}	40.76^{b}	1.72	NS	NS	NS	NS
MY, kg[4]	$9{,}920^{c}$	$10{,}500^{cd}$	$10{,}700^{cd}$	$11{,}884^{d}$	476.01	NS	NS	NS	NS

	Season[8]			
	I (n=52)	II (n=51)	SEM	Overall
MY, kg/d[1]	38.50	37.25	1.35	NS
MY, kg/d[2]	37.16	38.44	1.58	NS
MY, kg/d[3]	38.88	37.91	1.22	NS
MY, kg[4]	10,977	10,525	337.24	NS

[1, 3] multiparous Holstein cows milked 3x/day from 3-63 (n=103)[1] and 3-150 (n=102)[3] DIM. [2] 102 multiparous Holstein cows milked 3x/day from 64-150 DIM. [4] 89 multiparous Holstein cows milked 3x/day during 305-d lactation. [5] Contrast A: I (Control) vs. II (bST Post) + III (bST Pre) + IV (bST Pre/Post). [6] Contrast B: IV (bST Pre/Post) vs. II (bST Post) + III (bST Pre). [7] Contrast C: II (bST Post) vs. III (bST Pre/Post). [a,b] Superscripts that differ indicate significance ($P \leq 0.05$) by non-orthogonal contrasts. [c,d] Superscripts that differ in rows indicate trend ($P \leq 0.10$) for non-orthogonal contrasts analyses. [8] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb). † $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$.

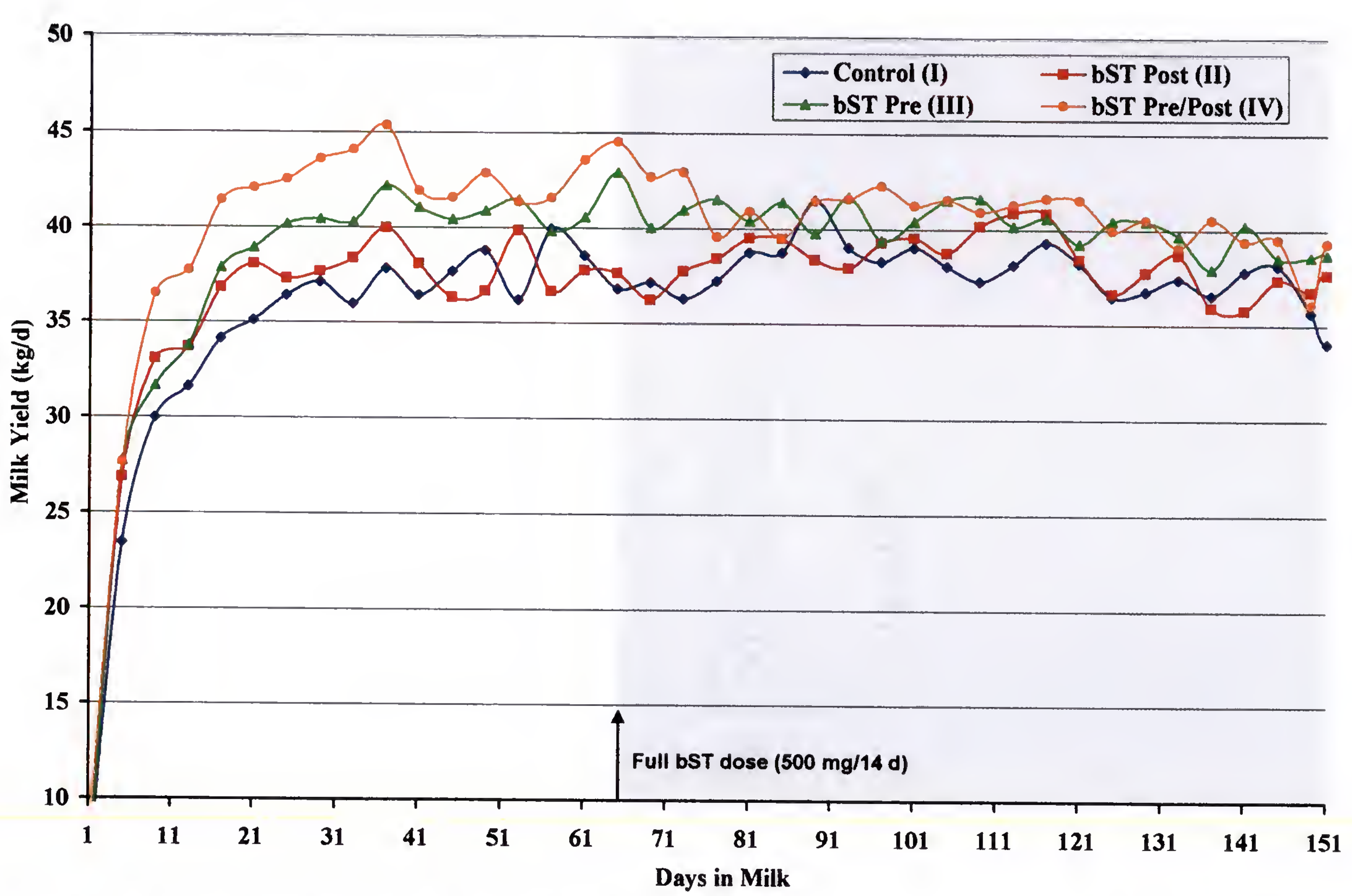

Figure 24. Least squares means depicting daily milk yield of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. Shaded area represents time during which full dose of bST was supplemented to all cows.

Table 44. Cubic regression coefficients that describe treatment effects on milk yield of Holstein cows during 3-150 DIM[1].

Treatment	β^2	$\beta1^2$	± SEM	$\beta2^2$	± SEM	$\beta3^2$	± SEM
Control (I)	26.24	0.40091	0.78237	-0.00404	0.00057528	0.00001206	0.00000233
bST Post (II)	32.28	0.21558	0.04190	-0.00197	0.00059061	0.00000482	0.00000238
bST Pre (III)	29.82	0.41246	0.03672	-0.00438	0.00051971	0.00001345	0.00000210
bST Pre/Post (IV)	33.96	0.38825	0.05000	-0.00472	0.00070512	0.00001581	0.00000285

[1] multiparous Holstein cows milked 3x/d.

[2] β' (Intercept), β1 (day), β2 (day*day) and β3 (day*day*day).

R^2 = 0.0541 (Control), 0.0204 (bST Post), 0.0556 (bST Pre) and 0.0247 (bST Pre/Post).

Mathematical model used: bST, season, bST*season, using cow (bST*season) as error term, with day to the third order as covariate.

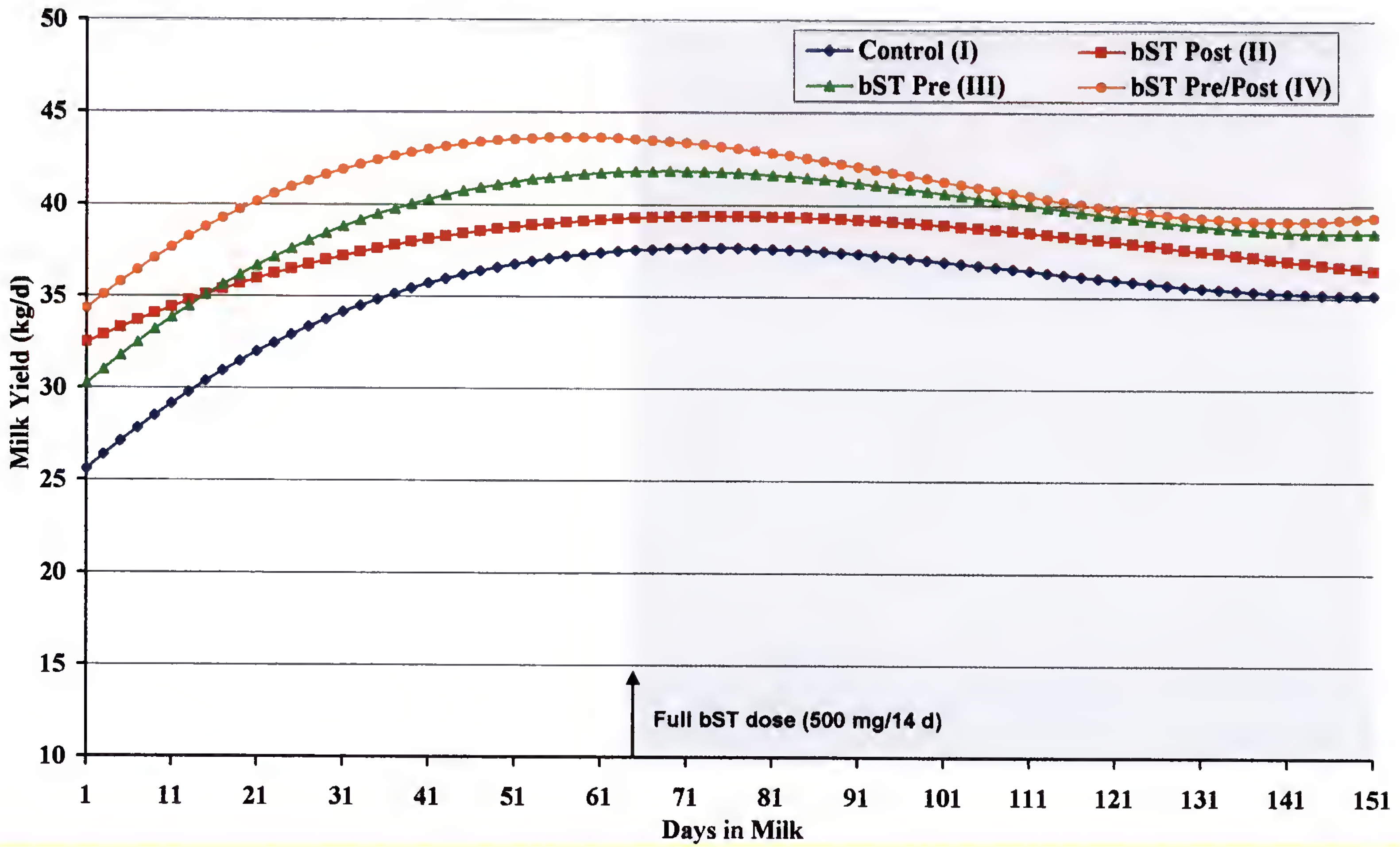

Figure 25. Cubic regression curves depicting mean daily milk yield of multiparous Holstein cows supplemented or not with bST during the prepartum and/or postpartum periods. Model: bST, season, and the two-way interaction of bST*season effect, using cow nested with bST*season as error, with day to the third order as covariate. Shaded area represents time during which full dose of bST was supplemented to all cows.

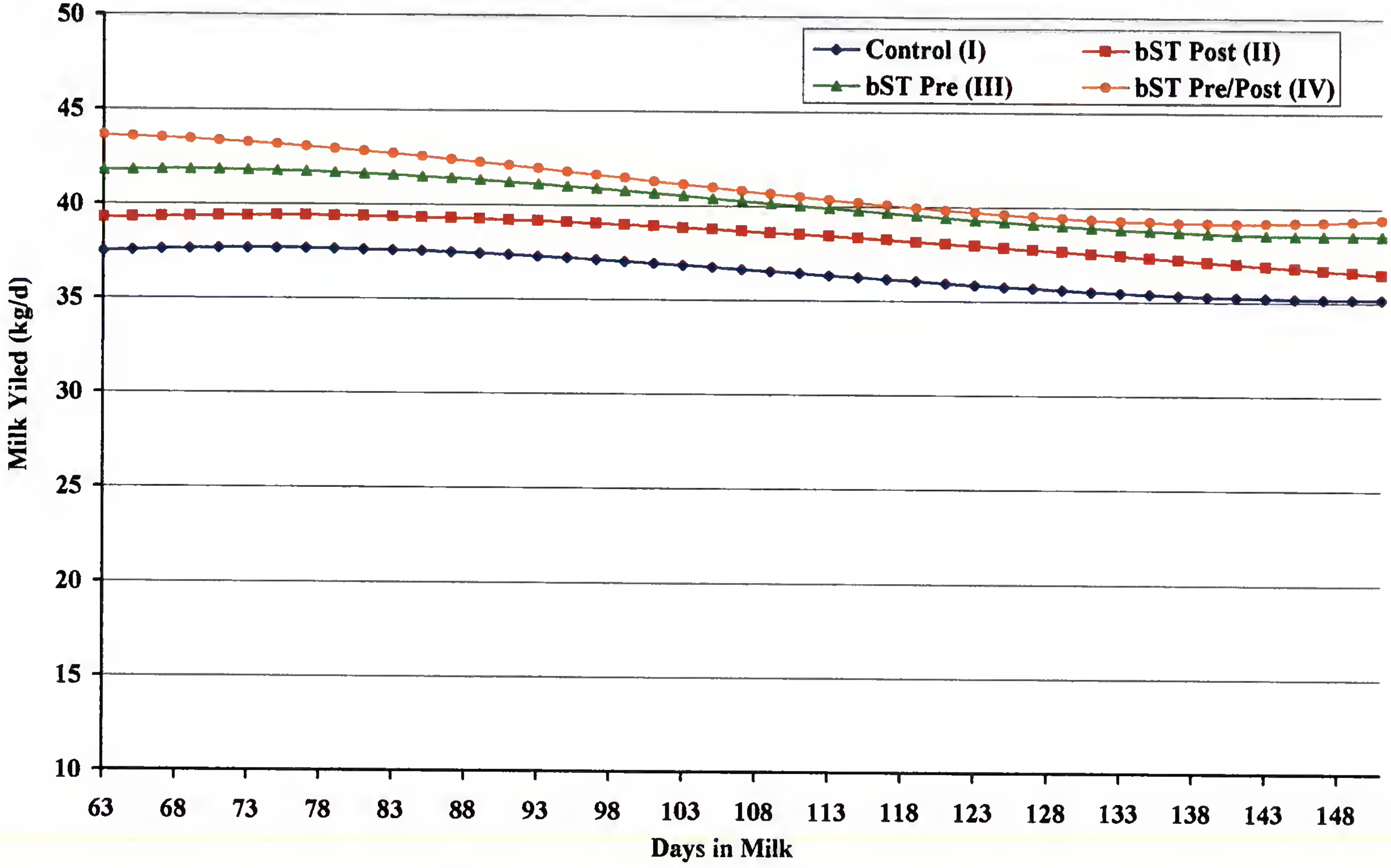

Figure 26. Cubic regression curves depicting mean daily milk yield (MY) of multiparous Holstein cows supplemented or not with bST (500 mg/14 d) from 64-150 DIM. Model: bST, season, bST*season effect, using cow(bST*season) as error, with day to the third order as covariate.

Table 45. Least squares analyses of variance for percentages of milk fat and milk protein and somatic cell count (SCC) in milk of Holstein cows from 3-63 DIM[1].

	Milk Fat				Milk Protein				SCC			
Source	Df[2]	MS[2]	F[2]	Pr > F[2]	Df[2]	MS[2]	F[2]	Pr > F[2]	Df[2]	MS[2]	F[2]	Pr > F[2]
bST[3]	3	1.19	0.41	0.7452	3	0.82	0.62	0.6019	3	5911604	1.14	0.3387
Season[4]	1	13.22	4.56	0.0354	1	23.19	17.57	0.0001	1	11333950	2.18	0.1433
bST*Season	3	0.43	0.15	0.9303	3	0.53	0.40	0.7514	3	8981038	1.73	0.1670
Cow (bST*Season)[5]	95	2.89	2.81	0.0001	95	1.27	5.53	0.0001	95	5200927	5.68	0.0001
Day	1	31.30	30.42	0.0001	1	103.84	451.58	0.0001	1	10486060	11.45	0.0007
Day*Day	1	3.84	3.74	0.0534	1	71.80	312.26	0.0001	1	15531193	16.97	0.0001
Day*Day*Day	1	0.16	0.16	0.6860	1	17.47	76.01	0.0001	1	14450259	15.78	0.0001
Error	1376	1.02			1481	0.22			1481	915466		

[1] 103 multiparous Holstein cows milked 3x/d.

[2] df (degrees of freedom), MS (Mean square), F (F-Statistic) and Pr>F (Probability).

[3] Treatments: I (Control), II (bST Post), III (bST Pre) and IV (bST Pre and Post).

[4] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb).

[5] Error term for sources above it.

Table 46. Least squares means and SEM for percentages of milk fat (FAT) and milk protein (PROT), and somatic cell count in milk (SCC) of Holstein cows for treatments and season of calving.

	Treatments					Comparisons			
	Control (n=26)	bST Post (n=25)	bST Pre (n=27)	bST Pre/Post (n=25)	SEM	Overall	A[2]	B[3]	C[4]
Variable						-----------P-----------			
FAT, %[1]	3.82	3.78	3.85	3.72	0.08	NS	NS	NS	NS
PROT, %[1]	2.90	2.83	2.80	2.80	0.05	NS	NS	NS	NS
SCC, x1000[1]	512.92	608.51	312.35	522.33	119.12	NS	NS	NS	†
	Season[5]								
		I (n=52)	II (n=51)		SEM	Overall			
FAT, %[1]		3.70	3.89		0.06	*	---	---	---
PROT, %[1]		2.96	2.71		0.04	**	---	---	---
SCC, x1000[1]		576.99	401.07		84.25	NS	---	---	---

[1] 103 multiparous Holstein cows milked 3x/day from 3-63 DIM. [2] Contrast: I (Control) vs. II (bST Post) + III (bST Pre) + IV (bST Pre/Post). [3] Contrast: IV (bST Pre/Post) vs. II (bST Post) + III (bST Pre). [4] Contrast: II (bST Post) vs. III (bST Pre/Post). [a,b] Superscripts that differ indicate significance ($P \leq 0.05$) by non-orthogonal contrasts. [5] Cows were grouped into two calving seasons, I (Aug-Oct) and II (Nov-Feb). † $P \leq 0.10$; * $P \leq 0.05$; ** $P \leq 0.01$.

CHAPTER 5
DISCUSSION

The main goals of this study were to determine if supplementation of low-doses of bST during the transition period and/or during early lactation would cause greater milk production of dairy cows without changing its composition, and to evaluate if productive changes occurred without adverse effects of health and reproductive status and were associated with the improvement in metabolic adaptations (changes in body weight, body condition score, hormones, growth factor, mineral and metabolites) and liver function during the transition period.

Comparison of milk yields (MY) across all treatment (TRT) groups showed that only when bST was supplemented during both the prepartum and postpartum phases of the transition period and also during early lactation was there a measurable positive and significant impact on milk production during lactation (Table 43). Compared to controls, cows on this regimen (TRT IV, bST Pre/Post) showed an 18.7% increase (6.51 kg/d) in mean daily MY during the first 63 DIM. Following the initiation of the full dose of bST (35.7 mg/d; 500 mg/14 d of POSILAC®) at 64 DIM, the relative difference in milk production between control and TRT IV group of cows decreased but a positive difference still existed but it was sustained to a lesser degree. Actual milk yield during the 64-150 DIM still differed, and in fact, MY of TRT IV cows, those supplemented both prepartum and postpartum, was 12.8% (4.64 kg/d) greater than non-supplemented controls (Figure 25).

Analysis of the actual milk yield for the whole lactation [actual MY(305-d)] adjusted for previous lactation MY indicated that differences observed during the first half of the lactation tended to continue throughout the second half of the lactation (>150 DIM), but as occurred during the 64-150 d time period the difference was less. Nonetheless, over the 305-d lactation cows on TRT IV produced 19.8% (6.44 kg/d; 1,964.1 kg/305-d) more milk than non-supplemented controls.

Results of current study did not agree wholly with several previous studies. Indeed, results that indicated there was greater milk production during early and later lactation contrast with those where greater doses of bST were supplemented during the transition period (Eppard et al., 1996). Importantly, current results did agree with a number of studies conducted at the University of Florida that used supplementation of lower amounts of bST (Garcia-Gavidia, 1998; Gulay et al., 2003b, 2004). In the study of Eppard et al. (1996), supplementation of 500 mg of bST beginning at 28 d prior to calving until approximately 14 d postpartum did not result in a significant increase in MY during early lactation. In fact, rather than causing an increase, there was a 16% decrease in daily MY of the supplemented cows. The authors attributed their failure to increase milk yield to increased health problems of the supplemented cows; problems that clearly were not related to direct effects of bST supplementation.

In an early study, Garcia-Gavidia (1998) evaluated effects of prepartum and/or postpartum supplementation of bST, using multiparous Holstein cows supplemented with 5.1 mg/d of bST beginning at 3 wk prepartum through 65 DIM. Supplemented cows (prepartum and postpartum) had a numerical, but not a significant increase in daily MY. Milk production of supplemented cows was about 4.2 % greater than non-supplemented controls (39.27 kg vs. 37.69 kg/d). In subsequent studies conducted by Gulay et al. (2003b, 2004), positive and much greater increases in MY were observed. In their studies, supplementation of bST was the same amount as used in the current experiment (10.2 mg/d of bST; 0.4 mL/14d of POSILAC®), and supplementation began ~21 d prepartum and continued through 42 DIM (Gulay et al., 2003b), or 56 DIM (Gulay et al., 2004). In the first study (Gulay et al., 2003b), bST supplementation caused a numerical increase in MY; supplemented cows produced an average of 2.4 kg/d more milk than controls during the early postpartum period (42 DIM). From 42 through 100 DIM, a period where no bST was supplemented to any cow, the numerical increase decreased as lactation progressed.

In the second study of Gulay et al. (2004), increases in MY were significantly greater than for non-supplemented controls. In addition to the prepartum, postpartum and early lactation supplementation of bST, all cows were supplemented with a full dose of bST (35.7 mg/d; 500 mg/14 d) beginning at 56 DIM and this was continued through the end of the lactation. During 0-42 and 60-150 DIM, the different amounts of bST supplemented caused increases of 3.9 and 3.3 kg/d, respectively compared to the controls. Over the 305-d lactation, cows supplemented with low dose of bST prepartum, postpartum and during early lactation tended to produce more milk than controls (+ 5.9%, 10,071 vs. 9,512 kg).

In the current study, cows supplemented only during the postpartum period (TRT II, 0-63 DIM) or only during the prepartum period (TRT III, -21 d until calving), showed increases in milk production compared to the non-supplemented control cows, but the small increases observed were not significant during 3-63, 3-150 DIM, or during the 305-d lactation (Table 43, Figure 24). During 3-150 DIM, the numerical increase in daily MY of these groups was about 5% greater than controls (1.56 and 1.89 kg/d for TRT II and III, respectively, Table 43). These tendencies also were reflected throughout the whole lactation period (305-d), where numerical increases of 5.8 and 7.8% were seen for these two treatments. These results lead to the conclusion that in addition to the postpartum injections, the prepartum supplementation beginning at about 21 d prepartum were necessary to stimulate the significant positive response with regard to the increase in daily MY, even though only 2 or 3 supplementations occurred prepartum. This means that prepartum and postpartum injections seemed to have an additive effect on milk production response.

Results obtained during the current study for milk yield response to bST-supplementation agree, in general, with many published results. In a study conducted using 156 multiparous Holstein cows, Bachman et al. (1992) observed that daily bST supplementation (25 mg/d) beginning at 21 through 7 d prepartum did not positively impact milk production during the subsequent lactation. In their experiment, bST-supplemented and control cows produced 28.4 and 29.5 kg/d of 3.5% FCM during the 305-d lactation. Similarly, in the current experiment the 305-d lactation MY of cows on TRT III (prepartum bST) did not differ from controls, although a numerical increase in daily milk yield was observed (35.08 vs. 32.52 kg/d; Table 43).

In a subsequent study conducted with 34 multiparous Friesian and Friesian-Jersey dairy cows, prepartum supplementation of bST also did not exert a positive effect on subsequent milk production (Law et al., 1994). In their experiment, cows supplemented with a single bST dose (640 mg) at 7 d before calving did not produce more milk than controls during early lactation. When 43 multiparous Holstein cows were supplemented with a high dose of bST (500 mg/14d; 35.7 mg/d) beginning 28 d before expected calving (~3 injections), milk production was not increased during the first 42 d of lactation, although again there was numerically but not significantly greater (7-10%) increase in average milk production (Putnam et al., 1999). Overall, these studies indicated that bST supplemented only during the prepartum period generally was not effective in causing a significant increase in daily milk production during early lactation.

In contrast to results obtained when cows were supplemented with full lactation dose of bST during the prepartum period, there have been more uniform and positive results across studies where low doses of bST were supplemented only during postpartum period. In fact, results of current study agreed with results of studies where low doses of bST were supplemented only during the postpartum period. For example, in a study conducted using multiparous Holstein cows, Stanisiewski et al. (1992) observed that supplementation with low dose of bST from 14-60 DIM tended to cause an increase in daily 3.5% FCM compared to controls. In their study, cows given 5 or 14 mg/d of bST produced 1.2 and 1.3 kg/d more milk during the 46-d supplementation period. Also, when cows were supplemented with the greater doses of bST (500 mg/14 d; 35.7 mg/d) during the early postpartum/lactation period (Santos et al., 1999; Moallem et al., 2000), there were significant increases in daily MY of supplemented cows compared to controls. In the first study (Santos et al., 1999), supplementation of primiparous (n=8) and multiparous (n=24) Holstein cows beginning 5 d postpartum through 90 DIM, significantly increased the amount of milk produced during the first 45 DIM (3.2 kg/d).

During the whole experimental period, the mean increase was 1.8 kg/d. In another study the same amount of bST supplementation (Moallem et al., 2000) from 10-150 DIM caused a significant increase in MY (5.4 kg/d) during the whole experimental period.

These increases in MY can be attributed to the greater amounts of bST given during lactation, and they also demonstrate that bST supplementation effectively increases MY, even during the very beginning of the lactation when cows are most likely in negative energy balance. This indicates that the cows must be drawing more extensively from their body reserves and/or produce milk more efficiently.

In addition to bST effects on milk production, possible effects on milk composition were evaluated. For this evaluation milk fat and milk protein percentages and somatic cell counts (SCC) were measured and data evaluated. Among all experimental groups, including the high-yielding group (TRT IV), no differences in the component percentages or SCC were observed (Table 45). This indicates that the lower amounts of bST supplementation during the prepartum and early lactation periods can significantly increase milk production without affecting its composition or quality, as measured by SCC. In fact, the average percentage of fat in milk produced by cows in all TRT groups were within the range expected for healthy Holstein cows (2.4-5.5%). The average percentage protein (2.83%) was only slightly less (-0.37 %) than expected (3.2%) during established lactation (Table 46; Goff, 2002). Overall, the percentages were similar to those observed in studies that used supplemental bST during the transition period or early lactation (Moallem et al., 2000; Gulay et al., 2004). The milk protein percentages observed were 2.86 and 2.92%, respectively in these two studies.

Despite the failure to detect a significant TRT effect, there was a significant effect of calving season on percentages of fat and protein in milk, but not on SCC (Table 45). Cows that calved during the hotter months (August-October; SEA I) had lower fat percentage (-0.19%) and higher protein percentage (+0.25%) than those cows that calved during the cooler months (November-February; SEA II). The changes in milk fat and protein percentages during the two calving seasons may be associated with slight nutritional differences in feed ingredients fed to cows during each period (Bachman, 1992; Badinga, 2000; Hanigan et al., 2001), and/or due to effects of photoperiod length (Stanisiewski et al., 1985; Phillips and Shofield, 1989; Aharoni et al., 2000; Dahl et al., 2000; Miller et al. 2000), which differed significantly for the two seasons.

Bachman (1992) described some nutritional factors responsible for altering the percentage of fat and protein in milk. Milk fat percentage is influenced positively by greater ruminal molar percentages of acetic (acetate) and butyric (butyrate) acids, but is negatively affected by propionic (propionate) acid, particularly when propionate exceeds 25 molar percent. A linear decrease in milk yield and an increase in milk fat percentage occurs as the ruminal acetate:propionate ratio increases to 2.2. However, an increased ratio of acetate:propionate likely is not the reason for the decrease in milk fat observed during SEA I, because milk volume was not affected during either season (Table 43).

The nutritional limitations for the production of protein are amounts of limiting AA (non-essential and essential [tryptophan, phenylalanine, methionine and histidine; Hanigan et al., 2001]), and low DM intake and/or energy content in the diet. In the current study there was a 0.25% increase in milk protein percentage during SEA I that was accompanied by a numerical, but non-significant increase in MY during the same months as in SEA II (Tables 43 and 46). Because MY during both seasons did not differ, it is unlikely that changes in available energy to produce milk differed and is the cause of the slight increase in milk protein percentage observed during SEA I. Of course, there could have been a slight decrease in essential AA in the diet fed during SEA II, which could have limited the ability of mammary cells to synthesize proteins, which in turn would result in a decrease in the protein content of the milk (Hanigan et al., 2001). While this is possible, it would have been an unexpected finding. Neither AA composition of diet, feed intake or other factors that could have resulted in slight changes in nutrient intake were evaluated critically and, in any case, the difference in protein percentage was not great.

An interesting possibility to explain, at least in part, the seasonal differences in milk component percentages, especially milk fat, is photoperiod effects on cows during the two different calving seasons. Some authors observed that artificial or natural increase in day length during early or established lactation or during the last 21 d prepartum can impact the amount of milk and constituents produced, but affect mainly the percentage of fat in milk. For example, in the study conducted by Stanisiewski et al. (1985) in Michigan (latitude 42° N), multiparous dairy cows (Holsteins, Jerseys and Brown Swiss) that were exposed to artificial light (16 to 16.5 h/d) beginning at 8 wk postpartum, produced 2.2 kg more milk with 0.16% less fat than herd controls that received 9-12 h of natural light during several weeks of lactation. In a subsequent study conducted in Wales, UK (latitude 53° N), Phillips and Schofield (1989) observed that British-Friesian cows supplemented with artificial light (8 h natural daylight + 10 h of artificial light + 6 h of dark), beginning at about 4-6 wk postpartum and continuing for 12 wk, produced the same amount of milk as controls, but milk fat percentage was about 0.18% less.

Aharoni et al. (2000) observed that changes in day length that were controlled by providing supplemental natural light before the initiation of the lactation affected total milk yields and percentages of components in milk produced by dairy cows in Israel (latitude 32° N). In their study, 28,029 test day milk records of 2,029 dairy cows collected during a 3-year period were evaluated. These records were analyzed to establish the influence of day length before parturition (DLP, beginning -21 d before calving) on milk variables. They observed that the increase in DLP significantly decreased MY and percentage of fat and protein in milk during the subsequent lactation. Dairy cows that had the shortest daylight length before calving produced 1.9 kg/d more milk during the subsequent lactation, and that the difference in milk fat percentage between seasons was 0.28%.

Besides the retrospective analysis of cow records, results of field trials also demonstrated similar effects of prepartum photoperiod on milk constituents and yields. In a study conducted by Miller et al. (2000) using 34 multiparous Holstein cows in the State of Maryland (latitude 39° N), they observed that cows exposed to long-day photoperiod (LDPP, 16h light and 8h dark) during the 60 d dry-period, produced less milk than those exposed to short-day photoperiod over the same time period (SDPP, 8h light and 16h dark). However, milk composition was not changed, but milk production increased 3.2 kg/d during the first 16-wk of lactation.

The biological mechanisms that explain changes due to photoperiod are not completely understood, but may include changes in feed intake and in secretion of hormones. For example, a strong possibility is that changes in concentrations of prolactin (PRL) that result from changes in photoperiod are involved. Dahl et al. (2000) summarized information on effects of photoperiod on concentrations of PRL in plasma. A common observation across many studies is that the natural or artificial increase in day length leads to an increase in concentrations of PRL in plasma of dairy cows. Because of the importance of PRL for lactogenesis (Head, 1999), it has been speculated that the increase in PRL concentrations during the time of lactogenesis (stages I and II) likely is involved, if not wholly responsible for the MY responses observed in dairy cows under long-day photoperiod regimen, and as a consequence the changes in concentrations of milk constituents. In fact, in the experiment of Miller et al. (2000), plasma concentrations of PRL in LDPP cows were greater (11.7 ng/mL) than in the SDPP group of cows during the prepartum time period; however, they did not observe changes in milk constituent percentages. Although PRL is necessary for lactogenesis and mammary cell differentiation during the prepartum period, PRL is not galactopoietic when given during lactation (Plaut et al., 1987). In their study, when PRL was supplemented exogenously to dairy cows during lactation either before or after peak milk production, no increase in milk production was observed.

In the current experiment, dairy cows were managed on the University of Florida dairy located at latitude 29° N, and based on historical data (Weather Channel® Website, 2004), they were exposed during the dry period and early lactation to 13 h and 10 min, 12 h and 19 min, and 11 h and 25 min of daylight during August, September and October (SEA I). During November, December, January and February (SEA II), daylight exposures were decreased to 10 h and 40 min, 10 h and 16 min, 10 h and 29 min, and

11 h and 09 min during the four months. The difference in daylight length between August (month with longest daylight period on average during the experiment) and December (month with the shortest daylight period on average) was 2 h and 54 min, or nearly 3 h. Compared to previous studies cited, experimental cows were not exposed to the amount of daylight that most authors used (~16 h), nor did exposure decline to less than 10.5 h of dark, so it cannot be assumed that the photoperiod length was an important reason for changes in milk composition (fat percentage). However it could have been one of a series of factors that summed together provoked the effects observed.

Among other changes that differ in cows during the two calving seasons is the increase in concentrations of NEFA in plasma during the postpartum period in cows that calved during the cooler months (SEA II ; Table 29). About one-half (molar percent) of the fat present in milk (milk fatty acids) originates from the *de novo* synthesis of fatty acids in mammary epithelial cells, in the form of short-chain (4 to 8 carbons) and medium-chain (10 to 14 carbons) fatty acids. The other one-half is composed of long-chain fatty acids (>16 carbons) derived from the uptake of circulating lipids; fatty acids of 16 carbons originate from both sources (Shennan and Peaker, 2000). The major source of carbon for the *de novo* synthesis of fatty acids in the ruminant mammary gland is acetate produced by rumen fermentation. In addition, β-hydroxybutyrate produced by the rumen or omasal epithelium from absorbed butyrate provides one-half of the first four carbons used in the *de novo* synthesis. Preformed fatty acids taken up by the mammary gland and directly used for milk fat synthesis are derived from circulating lipoproteins and NEFA that originate from absorption of lipids from the digestive tract and from mobilization of body fat reserves. Typically, lipolysis and mobilization of body fat accounts for less than 10% of the fatty acids present in milk fat; however, when cows are in negative energy balance, the contribution from mobilized fatty acids increases in direct proportion to the extent of the energy deficit, and the proportion of specific fatty acids in the milk fat changes to favor the longer chain acids (Bauman and Griinari, 2003). This could have happened in cows that calved during SEA II. Although the difference in fat percentage of milk produced during the two calving seasons was small (5.1%), the increase in plasma NEFA concentrations was fairly large (31.8%) during the period of NEB that lasted at least 28 DIM (Table 29; postpartum measurements of NEFA concentration in plasma). This may have contributed to the increase in milk fat.

With regard to the quality of the colostrum produced by cows in this study, as evaluated by content of immonoglobulins, observations were similar to those for milk components. Overall, bST supplementation did not affect content of the immunoglobulins in colostrum, but there was a significant SEA effect such that cows that calved during SEA I (hotter season) had a 22.7% greater (20.36 mg/mL) concentration of total proteins in colostrum than those that calved during SEA II. Despite these differences, colostrum produced during both seasons was of high quality (>50 mg/mL; Shearer et al., 1992; Hafez, 1996), by this criterion (Table 38). In fact, the pattern of immunoglobulin concentrations in colostrum during each season agreed with previous observations of Shearer et al. (1992), and partially to those reported by Hafez (1996). Both studies were conducted in Florida. In the study of Shearer et al. (1992), colostrum samples collected from 2,045 cows that calved in Florida were obtained at first milking and were evaluated for total protein (immunoglobulin, Ig) over a period of 2.5 yr (1983-1985). Results showed that cows that calved during the hotter months of the year (August and

September) had the greatest concentrations (~ 25 mg/mL) of any other calving month, similar to results during current study, whereas lowest concentrations were for calvings during December (~12 mg/mL) and April (~7.5 mg/mL). The absolute concentrations differed because measurements of Shearer et al. (1992) were made with samples at body temperature (37°C) and not at room temperature (25°C), as recommended and used in study of Hafez (1996) and the current study.

In the study conducted by Hafez (1996), a total of 961 colostrum samples were collected during the cold (November through February), warm (April, March, September and October) or hot (May through August) seasons of the year over a 6 yr time period (1989-1995). Mean immunoglobulin concentration obtained by a colostrometer across all samples was 77 mg/dL. An interesting observation was that immunoglobulin concentrations of cows within the same seasons often differed greatly. Nonetheless, the results of this study differed from observations of Morin et al. (2001). In their study, colostrum immunoglobulin concentrations of 75 multiparous Holstein cows in Illinois were greater in the autumn (193 ± 26 mg/mL; September through November) than in the summer (168 ± 39 mg/mL; June through August). They attributed difference to effects of heat stress on health and strength of the newborn calves. Shearer et al. (1992) and Hafez (1996) speculated that during the months of August and September in Florida, when there are strong effects of heat stress (average environmental temp of 32°C and high humidity), calves were less vigorous, and therefore less likely to nurse their dams, resulting in greater concentrations of immunoglobulins in colostrum at first machine-milking. This may have been responsible, in part, for differences observed in current and previous studies. Clearly, there are many other factors such as volume of colostrum produced, water and feed intake of the dam, parity of cow among others that that also can affect absolute concentrations of immunoglobulins and many of these were not considered in the current or previously published studies. An important observation by Hafez (1996) was that concentrations of many other important constituents in colostrum change in a similar pattern to changes in immunoglobulin concentrations. Thus, because the concentrations of immunoglobulins generally tended to be high, this indicates that these other constituents also were likely to be high in colostrum produced by cows even though seasonal differences existed during current experiment.

Another important objective of this study was to evaluate physiological changes that occur as a consequence of bST supplementation, during both the transition period and early lactation, that were associated with and supported greater milk production. During the prepartum period, bST-supplemented cows experienced a slight increase in BW and BCS (Figures 5 and 6), and this differed from non-supplemented control cows. Furthermore, following parturition (0-63 DIM), there was a significant decrease in BW across all TRT groups, as would be expected following expulsion of calf, fetal membranes and fluids. The sharpest and only decrease in BW occurred during the first 28 DIM, where losses in BW relative to pre-calving BW, were 13.84, 12.93, 14.05 and 12.12% for cows of the four TRT groups. This amounted to mean BW losses of 100.6, 94.9, 103.8 and 91.1 kg for these cows. During the same time period (28 DIM), the BCS losses relative to pre-calving BCS were 0.51, 0.49, 0.46 and 0.42 pt. or 14.48, 14.41, 13.25 and 12.10% (Tables 11 and 20). After this critical early lactation phase, all experimental cows maintained their BW or initiated a slow, but steady recovery in BW as lactation progressed (Figure 5). The BCS recovery began at about 9 wk after calving for cows on TRT I and IV, but did not occur until about 14 wk (~100 d; Figure 6) for cows on TRT II and III.

When the weight of the calf and the utero-placental unit and fluids lost during parturition were taken into account, the losses of body tissues that occurs during this period (adipose and muscular; Bauman and Vernon, 1993) were minimal. The average weights of the newborn calves (CLFWT) of cows on TRT I, II, III and IV were 40.36, 37.90, 39.03 and 39.15 kg, respectively, and they did not differ among TRT groups (Tables 37 and 38). It is likely that about 25-30 kg of utero-placental tissues and fluids also were lost during parturition (Bell, 1995); and therefore, it can be estimated that about 30 kg of body tissue were lost across all TRT groups during the first month of lactation. In fact, if 1 pt. body condition loss corresponds to a 56 kg of BW loss (Otto et al., 1991), the BW loss estimated by changes in BCS across all TRT groups during the first 28 DIM was of about 26 and 31 kg of BW, respectively, which is similar to BW loss estimates calculated. This indicates that BCS measurements recorded during the study were accurate and adequately represent real physical changes of the cows.

Compared to the BW at 28 DIM, the means observed at 63 DIM indicated that mean gains in BW of 15.48, 15.04, 6.36 and 4.05 kg were realized for cows on the four treatments. At 150 DIM, mean BW of cows on the four treatments were 66.61, 35.05, 43.00 and 36.73 kg greater than at 63 DIM (Figure 5). So, by mid-lactation all experimental cows, including those in the high-yielding group (TRT IV), had recovered the estimated amounts of tissues lost during early lactation, despite the substantial increase in MY observed in this group during the first half of the lactation (Table 43). The BCS changes during the postpartum period were similar across TRT and essentially paralleled BW changes. In fact, BW and BCS were highly correlated (Appendix B). At 63 DIM, the BCS change from 28 DIM was of +0.04, -0.01, -0.03 and +0.04 pt., respectively; a very slight change. At 150 DIM, the BCS gain of cows on the four TRT were 0.30, 0.18, 0.14 and 0.16 pt. (Figure 6). The average BCS of all groups of cows during 64-150 DIM was greater than 3.0 pt (Table 20).

Because DMI was not measured, it cannot be determined exactly when experimental cows achieved positive energy balance. In the study conducted by Gulay et al. (2003b), multiparous Holstein cows supplemented with low doses of bST (10.2 mg/d) from ~ 21 d prepartum through 42 DIM, achieved positive energy balance at about 42 DIM. Others (de Vries et al., 1999; Walters et al., 2002) observed that primiparous and multiparous dairy took until 72 or 85 DIM. Clearly, the time to achieve positive energy balance is affected by many factors, including but not limited to feed intake, quality of feed, milk production, environmental conditions and health status, among others.

The BW and BCS changes observed during the postpartum are one good indicator of the cows' ability to cope with the extra stimulus to produce milk because of bST supplementation. Results of the current study were similar to those obtained by Gulay et al. (2003b, 2004). In their first study, multiparous Holstein cows were injected with low doses of bST (10.2 mg/d) from ~21 d prepartum through 42 DIM, but then cows were not supplemented with the full dose of bST until 100 DIM. Supplemented cows better maintained BW and BCS than controls during the first 10 wk of lactation, and the BW and BCS losses of control cows were greater than bST-supplemented cows (8.5 and 5.6% for BW, and 6.8 and 3.9% for BCS). Numerically, but not significantly greater increases in MY by bST-supplemented cows were observed during the first 100 DIM. This indicated that supplementation minimized the mobilization of energy stores, possibly by stimulating increases in DMI (Vernon and Pond, 1997). In the second study (Gulay et al., 2004), in addition to supplementing the low dose prepartum and during early lactation, all cows were supplemented with the full dose of bST (500 mg/14d) beginning at 56 DIM throughout the end of lactation. They observed that bST supplementation did not cause a different pattern of change in BW and BCS during the first 14 wk of lactation; the BW and BCS losses and pattern of changes were similar for control and bST-supplemented cows, but the MY of bST-supplemented cows was significantly greater than non-supplemented cows during period when there was supplementation with the low dose of bST.

For SEA effects on BCS, cows that calved during SEA II (November-February) had higher BCS than SEA I cows during the prepartum phase (0.42 pt.; Table 11). Although not measured, it is likely that the effects of SEA on BCS also were due to changes in DMI during the prepartum period across all TRT groups, which led to increased body condition during the phase. This would be similar to the trend observed by McNamara et al. (2003). In their study, multiparous Holstein-Friesian cows fed either low [1.20 Mcal NE_L/kg of DM, silage:straw silage diet (SS)] or a high [1.47 Mcal NE_L/kg, grass silage +3 kg of concentrate (C)] energy density diets during the last 28 d prepartum. The C group consumed 2.5 kg/d more DM than the SS group during the prepartum period, and this led to a 0.12 pt. increase in BCS, whereas the SS group lost 0.09 pt. Besides the consumption of a diet with greater energy density, the increase in intake also led to an increase in BCS. Although differences in BCS due to SEA were seen in the current study, the prepartum BCS of experimental groups of cows in all TRT groups were in the recommended range of 3.25-3.75 pt. at calving (Wildman et al., 1982; Table 11).

Besides recording and evaluating changes in BW and BCS throughout the experimental period, changes in concentrations of insulin, somatotropin and IGF-I in plasma also were measured and evaluated in all experimental cows to determine the impact of low-doses of bST during the transition period, which is a period when great physiological adaptations occur. During the postpartum period concentrations of insulin in plasma of cows on the four treatments were 15.2, 21.4, 30.8, and 20.4% less than during the prepartum transition period in same groups of cows (Tables 13 and 24). The data depicted in Figure 7 clearly show that the decrease in insulin began at about 21 to 17 d prepartum and continued through ~8 DIM. Thereafter, concentrations slowly increased through 63 DIM. These trends in concentrations of insulin in cows as parturition approached and as lactation was initiated and then maintained are similar to those seen in many studies, even in studies that incorporated supplemental bST in cow management (Putnam et al., 1999; Vallimont et al., 2001; Gulay et al., 2004). In the study of Putnam et al. (1999), multiparous Holstein cows supplemented with the full dose of bST (500 mg/14 d) at -28 and -14 d prepartum had similar plasma insulin concentrations as controls during the transition period and early lactation. The mean concentrations of insulin in bST-supplemented cows, which did not differ from controls, were 0.72 and 0.47 ng/mL during prepartum (-28 d through calving) and postpartum periods (0-42 DIM). Using the same scheme, Vallimont et al. (2001) also detected a decrease of 57% in insulin concentrations in bST-supplemented cows during the prepartum and during the first week postpartum (0.93 and 0.40 ng/mL), which was similar to the decrease seen in control cows. Gulay et al. (2004) observed about a 45% decrease in mean concentrations of insulin in cows treated with lesser doses of bST (10.2 mg/d) during the last 3 wk prepartum and during the first 42 DIM (1.06 and 0.58 ng/mL). Thus, there is general agreement that bST-supplementation did not affect changes in insulin concentrations differently than occurs in non-supplemented cows. Decreasing concentrations appeared to be an essentially normal to expected physiological adaptation in the metabolism of carbohydrates in peripheral tissues of dairy cows during the transition period.

It was expected that concentrations of somatotropin in plasma that normally are seen during the phase would occur in both non-supplemental and supplemental cow, but to a different extent due to the extra bST supplemented, which would be expected to increase peripheral concentrations. In fact, concentrations of somatotropin did increase to a greater extent in supplemented cows (10.2 mg/d) during both the prepartum and postpartum periods. During the prepartum period, concentrations of somatotropin increased about 17% in cows on TRT IV compared to cows on TRT II (bST Postpartum), but did not differ from controls (Table 13). This was a surprise and is not understood. Overall, the differences in prepartum concentrations of supplemented groups compared to non-supplemented groups of cows were clear (~20%; Table 24). The ability of low doses of bST to increase concentrations of somatotropin in plasma during the prepartum period was described by Simmons et al. (1994). In their study, multiparous Holstein cows supplemented daily with 0, 5 or 14 mg/bST beginning at about 46 DIM through calving had 1.6, 6.5 and 22.7 ng/mL of somatotropin in plasma during these periods. In addition, supplementation of multiparous Holstein cows with daily doses of 25 mg of bST beginning at 21 d through 7 d prepartum increased mean concentrations in plasma of supplemented groups of cows by 183% (13.35 vs. 4.71 ng/mL; Bachman et al., 1992). Although absolute concentrations of somatotropin prepartum differed across these studies, it is clear that supplementing low doses of bST was effective at increasing circulating concentrations during the dry-period phase of the transition period.

Although some authors reported use of bST-supplementation during the postpartum phase of the transition period and throughout early lactation, reports of concentrations of somatotropin often were absent (Stanisiewski et al., 1992; Santos et al., 1999; Moallem et al., 2000). Only the studies by Gulay et al. (2003b and 2004) reported concentrations of somatotropin during the supplementation period and, as expected, bST-supplementation increased concentrations in plasma of cows during both studies. They reported average increases of 57 and 87% during the first 28 and 56 DIM. Overall, considering previous and current studies, it seems clear that low doses of bST-supplementation (10.2 mg/d) are enough to cause increases in somatotropin, and that these increases are accompanied by increases in milk production. Although increases observed in serum somatotropin may have direct affects in causing increased milk production, doubtless somatotropin also acts indirectly, and one likely mediator of this indirect action is IGF-I

Therefore, another objective of this study was to evaluate whether increases in somatotropin were accompanied by increases in concentrations of IGF-I in plasma. There is considerable evidence in the literature that somatotropin stimulates IGF-I production in dairy cows (Bauman and Vernon, 1993; Bachman et al., 1999). This effect of bST-supplementation was seen in the current study. For example, cows on TRT IV (bST Pre/Post) had greater mean IGF-I concentrations in plasma (19.4%) than controls during the prepartum period, but not in cows that were only injected postpartum (TRT II cows; bST Post; Table 13). Also, during the postpartum period and early lactation, TRT IV cows maintained greater mean concentrations of IGF-I than controls (26%), and concentrations of the IGF-I in TRT II cows also increased once bST-supplementation was started at calving; concentrations were about 16.3 % greater than in control cows, although this was not a significant increase Table 24).

In current study, plasma IGF-I concentrations were much less at calving than at time they were assigned to experiment about 3 wk prepartum (31.6, 45.8, 27.8 and 37.5%, less). However, as seen previously, at 63 DIM the mean concentrations of IGF-I of cows in the four treatment groups had increased and were 30.73, 60.9, 33.9 and 44.2% greater than observed at calving; but they still were 10.55, 12.77, 3.27 and 10.03% less than the initial value observed at the beginning of the experiment (21 d prepartum, Figure 9). As indicated, the trends seen for concentrations of IGF-I in plasma throughout the current study agreed with previous observations (Abribat et al., 1990; Vicini et al., 1991; Gulay et al., 2003b). In the study by Abribat et al. (1990), plasma IGF-I concentrations of Holstein females during the dry period, at 24 h postpartum, and at 54 d postpartum were 113.7, 44.7 and 92.7 ng/mL, respectively. In the study of Vicini et al. (1991), serum concentrations of multiparous Holstein cows at 21 d before and 14 d after the dry-off, and at 21 d postpartum were 89.1, 99.6 and 63.6 ng/mL. In addition, Vicini et al. (1991) also observed that supplemental bST (25 mg/d for 7 d) increased IGF-I concentrations during the phases they evaluated.

The physiological events responsible for the decrease in IGF-I concentrations in plasma of transition cows as calving approaches have been studied extensively in recent years (Thissen et al., 1994; Bauman, 1999; Breier, 1999; Kobayashi et al., 1999; Kobayashi et al., 2002; Butler et al., 2003; Radcliff et al., 2003). The IGF-I protein is produced by almost all body tissues; however, the majority of IGF-I (80-90%) present in blood of humans and animals, including ruminants (Yakar et al., 1999), is produced by the liver, which also contains an abundance of ST/GH receptors. These receptors confer the capacity to the organ to produce and secrete IGF-I under somatotropin stimulation (Bauman and Vernon, 1993).

In cattle, three types of GH receptors (GHR) are expressed by three promoters in the GHR gene: P1, P2 and P3. The P1 is liver specific and alternatively splices exon 1A onto the GHR mRNA (GHR 1A mRNA). The second and third promoters (P2 and P3) have constitutive activity in many tissues and alternatively splice exons 1B and 1C onto GHR mRNA (GHR 1B and 1C). The total amount of GHR in the liver partially determines liver IGF-I production in response to GH (Kobayashi et al., 1999).

The changes in the pattern of IGF-I concentrations in plasma of transition cows are directly related to the presence of total GHR mRNA in liver. In the study by Kobayashi et al (1999), the patterns of GHR and IGF-I mRNA expression were evaluated in liver obtained at 14 d prepartum, day of calving, and at 14 d postpartum from primiparous Holstein cows fed once daily to meet or exceed their daily requirements (National Research Council [NRC], 1989). They observed that there was a decrease in total GHR mRNA at parturition associated with a specific decrease in GHR 1A mRNA, but the amount of GHR mRNA 1B and GHR 1C mRNA did not change at parturition, whereas total liver IGF-I mRNA and blood IGF-I concentrations also were decreased at parturition.

In the study by Radcliff et al. (2003), patterns of liver GHR 1A and IGF-I mRNA expression, as well as profiles of blood metabolites were observed in multiparous Holstein cows (n=65) from 14 d prepartum until 14 d postpartum; these cows were fed to meet or exceed their nutritional requirements (National Research Council [NRC], 1989). The GHR 1A mRNA began to gradually decline at 2 d before parturition, was lowest 3 to 4 d after parturition, and then increased; however, it did not achieve prepartum levels by 14 d postpartum. Importantly, the total GHR mRNA was not affected by day the liver sample was taken and evaluated, as was observed for the specific GHR 1A in a previous study. The pattern of IGF-I mRNA expression was similar to the GHR 1A receptor mRNA expression, but occurred slightly later than the decline in GHR 1A mRNA; and changes in plasma concentrations of IGF-I were parallel to changes in liver IGF-I mRNA.

From these studies it can be concluded that the liver is the primary source of circulating IGF-I, that changes at the molecular level with regard to GHR in the hepatocyte are responsible for changes in IGF-I concentrations in plasma during the transition period, and that there is a strong relationship between the physiological stage and the metabolic adaptations around calving, as reflected by the decrease in IGF-I secretion. In fact, a recent report by Butler et al. (2003) showed that early postpartum cows subjected to a hyperinsulinemic-euglycemic clamp had increased GHR 1A and IGF-I mRNA expression in liver. Indeed, in the current study, concentrations of insulin and glucose in plasma were positively correlated with IGF-I concentrations during the postpartum period (Appendix B). Because bST-supplementation increased concentrations of IGF-I to some degree during prepartum and around calving, even when the typical decrease in concentrations of IGF-I was occurring and reduced amounts of mRNA for GHR 1A in liver would be expected, it appears that bST still was able to effect an increase in concentrations of IGF-I. This ability of ST would favor positive effects that would be expressed as increased milk production, especially at the initiation of the lactation.

Although actions of somatotropin, via effects on liver, doubtless are important, in addition to metabolic changes the amount and type of nutrients consumed by ruminants also impacts concentrations of IGF-I in the blood. It is well known that feed intake (DMI) also decreases as cows approach calving (Grummer, 1995). In a study designed to mimic the decrease in DMI seen in dairy cows right before calving, Kobayashi et al. (2002) observed a decrease in IGF-I concentrations in plasma and in IGF-I mRNA expression in the liver after feed restriction, but the amount of GHR 1A mRNA was not affected. This demonstrates that feed intake may be partially responsible for the decrease in IGF-I concentrations, but not of GHR mRNA receptor. Kriel et al. (1992) earlier made an interesting observation with regard to effects of nutrition on IGF-I concentrations in plasma of ruminants. In their study, castrated male lambs fed diets containing restricted metabolizable energy (ME) with or without restricted nitrogen intake, were infused with saline or glucose (i.v.) for 6 d, and challenged with a GH injection on d 5 of the experiment. In the first experiment that included restricted ME and nitrogen intakes by the lambs, no increase in IGF-I concentrations were observed in response to GH challenge in lambs that were infused with saline. However, if lambs were infused with glucose their IGF-I concentrations increased significantly. In a second experiment that included restricted ME, but not nitrogen, peak concentrations of IGF-I were significantly greater in the glucose infused than in saline infused group than seen in previous experiment in which nitrogen intake was restricted. This indicated that increased nitrogen supply in the diet and/or infusion of glucose re-establishes the IGF-I response to GH challenge as well as increasing IGF-I concentrations in plasma of ruminants, as Butler et al. (2003) demonstrated with dairy cows.

Based on the pattern of IGF-I concentrations in plasma of TRT IV cows (bST Pre/Post), it seems clear that the liver is sensitive to the supplementation of low doses of bST prepartum but only until 7-5 d before calving, after which concentrations of IGF-I begin to decrease (Figure 9). This change in sensitivity to bST occurs just when the decrease in GHR 1A mRNA expression is observed prepartum. It also seems that when IGF-I concentrations in plasma of prepartum transition cows are greater (TRT IV), bST-supplementation still is effective at maintaining concentrations of IGF-I greater until 7 d prepartum. When the IGF-I concentrations are lower, as for cows in TRT III, there were numerical increases in IGF-I concentrations with bST-supplemented, but they were not different from controls (Figure 9).

During the postpartum, the secretion of IGF-I in plasma in response to the increased somatotropin stimulation also seems to be dependent on the presence of GHR 1A. The mean concentration of IGF-I in TRT II cows (bST Post) did not differ from controls (Control) or TRT III (bST Pre) due to two possible reasons (Table 24). One is that concentrations of IGF-I in plasma of the supplemented cows were higher than in the non-supplemented group of cows only beyond ~15 d postpartum, and/or because IGF-I concentrations in the non-supplemented cows also increased as lactation progressed

(Figure 9 and 17). With regard to TRT IV (bST Pre/Post), the postpartum concentrations of IGF-I were maintained at a greater plateau than concentrations of cows in any other TRT group until ~15 DIM, and then began to increase as lactation progressed. Perhaps, the maintenance of a greater increase in feed intake was the cause of the differences in IGF-I concentrations and in productive response of TRT IV cows.

In addition to changes and trends in concentrations of hormones and IGF-I, similar evaluations were completed for effects of low doses of bST-supplementation on metabolites and calcium and their associations to support milk production. From evaluation of total calcium concentrations in plasma (Ca) prepartum and postpartum, it can be concluded that no negative or positive effects of bST supplementation were observed. The anionic diet fed during the prepartum period was effective in maintaining Ca levels during the whole experiment (21 d prepartum through 28 DIM; Tables 16 and 29). The only decrease in Ca observed during this measurement period was at the day of calving (Figure 10). Importantly, concentrations of calcium in plasma of cows in all TRT groups returned to prepartum levels immediately after parturition and all were within the acceptable physiological range of 9-12 mg/dL (Moore et al., 2000; Miles, 2001). These observations agree with reports in the literature that demonstrate the efficacy of anionic salts included in prepartum diets to formulate anionic diets. Feeding these diets allows cows to maintain satisfactory concentrations of calcium and thus reduces the risk of hypocalcemia postpartum as milk production rapidly increases and calcium needs rapidly increase (Horst et al., 1997; Goff and Horst, 2003).

One of the first studies to observe the efficacy of these anionic diets in maintaining calcium levels in the blood, and therefore to prevent or reduce incidence of milk fever during the transition period, was conducted by Block (1984). In one experiment, multiparous Holstein and Ayshire cows fed anionic diets beginning at 45 d prepartum experienced no cases of milk fever (MF) following calving, whereas 47% of cows in the group that consumed cationic diets had MF. The pattern of changes and the Ca concentration in plasma of cows fed anionic diets was similar to that seen in the current experiment.

For glucose, all experimental cows were able to maintain similar concentrations of glucose in plasma during the transition period and early lactation, even though concentrations decrease and are 4.78, 9.87, 9.48 and 6.85% less than mean prepartum concentrations for cows on the four TRT (Table 16 and 29). The pattern of changes in glucose concentrations during experiment was similar among TRT groups and also was similar to the patterns described for INS and IGF-I (Figures 7 and 9); correlation coefficients between glucose and insulin, and between glucose and IGF-I were positive (Appendix A and B). Across TRT groups of cows, the mean prepartum concentrations differed due to calving season (SEA; Table 16). Dairy cows that calved between November and February (SEA II) had 6.2% greater mean concentration of glucose in plasma than those that calved during SEA I (August-October). Although DMI was not measured, this small but significant difference in glucose concentrations may be associated with slightly greater feed intake by cows when the weather was cooler. Certainly, this would agree with findings for lactating cows.

Supplementation of low doses of bST during the prepartum and postpartum periods (through 28 DIM) did not affect the pattern of changes in mean NEFA across all TRT groups. As seen previously, concentrations peaked around calving (Table 16 and 29, Figure 12). The NEFA concentrations of cows on the four TRT during the postpartum period were 30, 41.39, 37.37 and 35.66% greater than the mean prepartum concentrations. Also, cows that calved during SEA II had greater concentrations of NEFA during both prepartum and postpartum periods than those that calved during SEA I. The increases were 27 and 31.8%. These increases also may have been associated with the feed intake, but contrast the results for glucose concentrations, which increased in cows during SEA II. Apparently, bST may have increased mobilization and peripheral concentrations of NEFA. Alternate possibilities for trends seen for NEFA are that there was greater mobilization of NEFA but they were not completely removed from the peripheral circulation and metabolized for energy, or stored in adipose again, or taken up and utilized and/or stored in the liver.

Although differences in concentrations of NEFA were detected both prepartum and postpartum, prepartum concentrations of β-hydroxybutyrate (B-HBA) were not affected by TRT and mean concentrations were similar for the two SEA (Table 16). However, during the postpartum period, cows supplemented with low doses of bST beginning at parturition (TRT II) had similar concentrations of B-HBA as non-supplemented controls and concentrations were greater in blood than cows in control and TRT IV groups (Table 29). During the postpartum, plasma B-HBA of cows on TRT I, II, III and IV were 73.1, 165.8, 139.3 and 97.3% greater than seen prepartum. Furthermore, cows that calved in SEA II had greater concentrations of B-HBA during the postpartum period than those that calved in SEA I (Tables 16 and 29). The pattern of changes in B-HBA (Figure 13 and 18) shows that the increases began a few days after the increase in plasma NEFA, but the rise was before calving (Figure 13). Correlation coefficients between B-HBA and NEFA were positive (Appendix A and B) indicating that mobilization of NEFA and increase in B-HBA were linked by the metabolism of NEFA; peak concentrations occurred about 7 d postpartum, after which they declined, except for cows in TRT II. Mean concentrations of B-HBA in cows on TRT II differed greatly, increasing throughout the 4 wk of lactation period in contrast to the gradual decrease seen in the other three TRT. Apparently, the difference in accumulation of B-HBA as lactation progresses in the groups of cows that were supplemented with bST during postpartum period occurs because of greater lipid mobilization, NEFA metabolism is reduced and/or there is incomplete metabolism of NEFA. Perhaps these differences in B-HBA and NEFA for the two postpartum bST-supplemented groups indicate that prepartum bST supplementation improved the metabolic pathways of lipid mobilization and utilization thus allowing complete NEFA metabolism and reduced B-HBA concentrations.

In general, studies that have used bST-supplementation as a way to improve metabolic adaptations during the transition period and to improve milk production obtained similar results for changes in concentrations and trends in glucose, NEFA and B-HBA in plasma. When multiparous Holstein cows were supplemented with full doses of bST at 28 and 14 d prepartum, no negative effects were detected on the concentrations of NEFA, B-HBA and on the accumulation of fat and triacylglycerol in liver of transition cows (Putnam et al., 1999). During the prepartum period, bST-supplemented cows had numerically lower NEFA and B-HBA concentrations in plasma, and during the postpartum no differences were observed. Concentrations of B-HBA in plasma during the current study were similar to experiment reported by Putnam et al. (1999) during both the prepartum and postpartum periods (about 8.1 and 11.0 mg/dL). When multiparous and primiparous dairy cows were supplemented only during the postpartum period (beginning at 5 d postpartum) with a full dose of bST (Santos et al., 2000), there were no effects of bST on concentrations of glucose (~50 mg/dL) or B-HBA (~9 mg/dL) during the first 45 DIM, but there were significant positive effects of bST on NEFA concentrations compared to non-supplemented cows (~500 vs. 387 µEq/L).

In the studies conducted by Gulay et al. (2003b and 2004), bST was supplemented using the same dose as used in the current study (10.2 mg/d) during the prepartum and postpartum periods (21 d prepartum through 42 and 28 DIM for the first and second studies). The bST did not affect concentrations of glucose or NEFA during the prepartum period; however, during the postpartum, no effects of bST were observed on glucose concentrations in the two experiments, but for NEFA there was a significant TRT effect during the first but not the second study. In both studies, prepartum concentrations of glucose and NEFA in plasma ranged from 66.3 to 71.6 mg/dL and 241.1 to 273.5 µEq/L, respectively. During the postpartum period, glucose concentrations were slightly less than seen prepartum in both studies, at around 60 mg/dL, than seen prepartum in both studies. During the postpartum period in the first study (Gulay et al., 2003b), concentrations of NEFA in supplemented and control groups were 579.8 and 470.0 µEq/L, respectively; whereas in the second study (Gulay et al., 2004), postpartum concentrations of NEFA were 634.2 and 582.4 mg/dL, respectively for supplemented and control groups. The increase in NEFA observed during the postpartum in the first study was attributed to increased lipolysis caused by bST supplementation, as described by Bauman and Vernon, 1993. Although similar effects of bST were observed in current study, concentrations of glucose and NEFA, but not B-HBA, were greater than reported by Gulay et al. (2003b, 2004). However, these and current studies did not measure feed intake, which may have affected concentrations of these metabolites.

In an experiment conducted by Doepel et al. (2002) using transition Holstein cows fed diets differing in amounts of net energy of lactation (NE_L) and crude protein (CP) during the last 21 d before expected calving they had similar concentrations of NEFA in plasma as in the current study. One of the experimental groups of cows (low energy/high protein) had peak concentrations of NEFA in plasma on the day of calving (1286 µEq/L). As lactation progressed, concentrations decreased to 422 µEq/L at 28 DIM. It is important to mention that their cows had a BCS of 3.19 during the prepartum period, therefore they were not overconditioned, which is a predisposing factor to high NEFA concentrations in blood (Grummer, 1993). Therefore, concentrations of B-HBA across all TRT groups of cows during the prepartum and postpartum periods were similar in both studies (Doepel et al., 2002; and current study).

A likely reason for the increase in B-HBA concentrations in plasma of cows on TRT II is because bST supplementation that began at calving caused an increase in the mobilization of NEFA from adipose tissue (Vernon and Pond, 1997). In fact, cows on TRT II had the greatest concentrations of NEFA in plasma during the period (961.07 µEq/mL; Table 29), even though it was not statistically different from all other groups. Because the ruminant liver does not have a limiting step regulating NEFA uptake (Drackley, 1999), increased mobilization likely caused an increase in the hepatic accumulation of fat, leading to increased ketogenesis, as observed by Gaal et al. (1983). Despite the increased concentrations of B-HBA, the mean concentration in TRT II cows was below 14.4 mg/dL (Oetzel, 2000), the concentration that indicates the cows are ketotic. Therefore, bST-supplementation did not cause the cows to become ketotic, although they were sub-ketotic, based on B-HBA concentrations in group II.

The effects of SEA of calving on concentrations of glucose prepartum, NEFA prepartum and postpartum, and postpartum B-HBA (Tables 16 and 29) can be interpreted relative to the increase in BCS observed prepartum in cows that calved during the SEA II (Table 11). The BCS of cows that calved during SEA II was greater BCS (0.42 pt.) than for cows that calved during SEA I, but the increase did not lead to cows becoming over-conditioned because BCS still was within the 3.25-3.75 pt. range recommended at calving (Wildman et al., 1982). The increase in BCS and concentrations of glucose are further indications that these cows consumed more feed than cows in SEA I. Apparently the greater feed intake was enough to maintain concentrations of blood metabolites slightly greater than cows that calved during the hotter seasons of the year (SEA I). In turn, they gained more body condition during this critical phase, similar to the observations in the study of Smith et al. (1997). Their experiment consisted of feeding multiparous groups of Holstein cows *ad libitum* or normal fed during the dry period with the intention to induce ketosis postpartum. The *ad libitum* feeding scheme caused an increase of 0.50 pt. in body condition immediately before calving compared to the normal fed controls. Even though concentrations of glucose were measured during their experiment, the number of samples obtained between -7 and +14 d postpartum (2) were insufficient to detect a statistical difference among treatment groups.

The greater availability of fat tissue in SEA II cows led to greater mobilization of NEFA around parturition, as observed by Rukkwamsuk et al. (1998). In their study, multiparous Holstein-Friesian x Friesian-Holstein cows that were overfed during 8 wk of the dry period (before parturition) had a four-fold increase in concentrations of NEFA at 0.5 and 1 wk after calving compared to cows fed restricted amounts of feed. In addition, concentrations of B-HBA were greater in overfed cows during wk 1 and 2 after parturition, but the differences were not significant. No BCS were reported in their study, but there was a greater numerical increase of BW in the overfed cows. In the current study, greater fat deposition in liver caused by the increased NEFA mobilization in the high BCS cows of SEA II apparently led to a greater ketogenic activity by the organ after parturition, but cows were not ketotic (<14.4 mg/dL of B-HBA).

It is clear at this point that supplementation of low-doses of bST influences the metabolic adaptations of dairy cows during the transition period. Therefore, it is important to investigate whether these important changes are associated with negative or positive effects on metabolism of fats and carbohydrates in the liver during this critical phase. To accomplish this, a subset of cows in each TRT group was evaluated in the current study at specific time points during the transition period. In general, results indicated that the lower amounts of bST supplemented during the transition period and early lactation did not negatively impact liver function with regard to metabolism of fats (Table 31). This agreed with interpretation of the metabolite data, especially for B-HBA. Numerous studies in the literature (Grummer, 1993; Drackley, 1999) have shown there were changes in fat deposition in liver (percentage of fat in liver [LFAT]) during the transition period, as observed by changes in all TRT groups (Table 33). However, changes seen were similar across TRT groups of cows evaluated. Across all biopsy days, greater fat deposition in liver occurred at d 14 postpartum (Table 33). For that biopsy day, there was a 72% increase of LFAT percent compared to prepartum liver samples (d –21).

Another important observation was that at the last biopsy (28 DIM), cows on TRT II, which had higher levels of NEFA and B-HBA in plasma than any other TRT group, also tended to have greater fat deposition (~57%) in the liver than other treatment groups (Table 34, Figure 19). These findings represent additional evidence that when bST is supplemented in low dose beginning at parturition through 63 DIM, it affects whole-body metabolism of fats, with effects reflected on hepatic metabolism during the period.

The triacylglycerol (TAG) content of the fat accumulated in the liver of experimental cows presented a similar picture as LFAT deposition (Table 33). No negative effects of TRT were observed for TRT I, III and IV, and the pattern of TAG accumulation, as well the differences, were similar as lactation progressed. In addition, percentage of TAG accumulation in liver was highly correlated with percentage of fat in the organ (LFAT) on wet basis (Appendix D), corroborating similarity of results. Similar to the observations for LFAT, TAG concentration in liver at d 14 postpartum was greatest of all samples obtained during the transition period. On that biopsy day, there was a 205% increase in TAG concentration compared to the biopsy samples obtained prepartum (Table 33). In addition, difference was significant with regard to TAG, although there only was a tendency for greater LFAT deposition in liver of TRT II cows on d 28 postpartum. For example, cows on TRT II had 137, 93.5 and 134% higher TAG in liver than cows on TRT I, III and IV, respectively on the last day of biopsy (d 28, Table 34, Figure 20).

In conjunction with quantification of LFAT and TAG in liver, the relative abundance of hepatic mRNA encoding *microsomal triacylglycerol transfer protein 1* (MTP), that also is related to liver metabolism of fats, was measured in liver samples collected. The MTP protein is a heterodimer constituted by a 59 kDa protein disulfide isomerase and a 97 kDa unique subunit that facilitates the assembly and secretion of apoB-containing lipoproteins [chylomicrons in the intestine and very-low density lipoprotein (VLDL) in the liver]. Its function is to transfer triacylglycerol (TAG), cholesteryl esters and phospholipids to a nascent apoprotein, and to assemble the VLDL in the rough and smooth endoplasmic reticulum. In humans, expression of MTP is increased and decreased by dietary saturated fatty acids and insulin, respectively (White et al., 1998). Because ruminants, including dairy cows, have an inherent decreased capacity to export TAG via VLDL (Pullen et al., 1990), mainly during the transition period, another goal of this study was to observe the pattern of MTP mRNA expression of experimental cows during this critical phase, and also to determine if the accumulation of fat in the liver when cows were supplemented with low doses of bST was positively or negatively correlated with MTP mRNA expression.

From the densitometry values, it was observed that bST-supplementation during the transition period did influence mRNA expression of MTP (Table 32). Throughout the whole transition period, cows on treatment IV (bST Pre/Post) had significantly lower MTP expression in the hepatocytes than cows on TRT I and II, but was not different from TRT III (bST Pre) group. In addition to overall treatment effects, there also was a day of biopsy effect on MTP mRNA expression. Biopsies collected at d 21 prepartum and at d 2 postpartum had greater amounts of MTP mRNA than at d 14 and 28 postpartum. At d 28 postpartum, mRNA expression observed was the lowest across all days (Table 33). When the comparisons of TRT groups were made within each day of biopsy, significant differences were detected at both d 14 and d 28 of lactation. At d 14, cows on TRT III had lower MTP mRNA expression than cows in groups I and II, but not from IV. At d 28, levels of mRNA in group III had increased and did not differ from any other group. The only difference detected was between TRT II and IV (Table 36, Figure 23). From these results, it can be concluded that TRT II cows, which had higher TAG levels in the liver at d 28, had upregulated MTP expression. Furthermore, cows in TRT IV had lower content of TAG than TRT II cows and lower MTP mRNA expression at the end of the transition period, even though overall, there was a negative correlation between TAG and MTP observed (Appendix D).

To date, no studies have been published that investigated the effects of low doses of bST-supplementation during the transition period on the hepatic deposition of fats and mRNA expression of MTP. However, studies with different objectives have been published that can give some insight into changes that occur in these measures. For example, a study designed to determine the contribution of feed intake depression prior to calving on the development of fatty liver (Bertics et al.,1992) reported that there was about a 93 % increase in percentage of liver lipid (total fat) at d 1 and 28 postpartum compared to d 17 prepartum. The percentages of fat in liver (dry matter basis) were 15.9 and 30.6% during prepartum and postpartum. For TAG, increases also were observed on d 1 and 28 postpartum (+227 and 279%) compared to prepartum levels; actual percentages on prepartum d 17, and postpartum d 1 and 28 were 7.1, 23.2 and 26.9%. In addition, cows that were force-fed during the last 15 d prepartum (mimicking the maintenance of feed intake), showed a 75% increase in TAG% compared to prepartum period. Among factors that likely were responsible for this decrease in extent of TAG increase, was prepartum DMI. In fact, DMI prepartum was most negatively correlated with increase in TAG deposition in liver. However, in the current study, concentrations of NEFA did not differ among TRT groups of cows during the prepartum and postpartum, and were not correlated with levels of TAG in liver.

Subsequently, Vazquez-Anon et al. (1994) conducted a study to monitor liver TAG before and after parturition, and to estimate relative contribution of depressed DMI and parturition to plasma NEFA concentrations and the development of fatty liver. At d 17 prepartum, dairy cows were fed diets *ad libitum*, and liver biopsies were conducted on d -17, -10, +1, +7, +14 and +21 from calving. In addition, blood samples were collected on d 17, 10, 5, 3 and 1 prepartum, and d 1, 7, 14 and 21 postpartum. Results indicated that prepartum DMI depression was not seen until d 2 before calving; plasma NEFA concentrations peaked on d 1 after calving (average 1014 μM/L). The initiation of hepatic TAG infiltration occurred at d 1 after calving; and the highest mean hepatic TAG level was 12% (DM basis) at d 21 postpartum. One of the conclusions was that TAG infiltration did not occur until there was an acute rise of NEFA at calving, similar to observations of current study. The peak concentrations of NEFA occurred between calving and 2 d postpartum (Figure 12), and the peak of TAG accumulation was at d 14 after calving (Figure 20). The slight difference in timing of these events observed in these two studies likely was because the number of biopsies and time they were taken relative to calving differed. Overall, trends were similar for the two studies and indicate that greater concentrations of NEFA, that indicate greater mobilization of lipids from adipose, led to greater accumulation of liver lipids and that DMI could modify this.

When multiparous transition cows were supplemented with full dose of bST (500 mg) at d -28 and -14 from calving (Putnam, et al., 1999), no effects of bST were detected on deposition of fat and TAG in liver of these cows. Mean percentages of fat and TAG in liver on wet basis at d -28, -3, 3 and 28 relative to calving were 4.57, 5.69, 9.25 and 10.84% and 0.25, 1.32, 3.86 and 3.84%. In the study conducted by Selberg et al. (2004), percentage of total fat and TAG in liver of transition Holstein cows used as the control group in an experiment that examined the effect of supplementation of calcium salts of CLA and *trans*-$C_{18:1}$ isomers on production and metabolic responses, were similar to levels observed in the control group (TRT I) of the current study. At d 2, 14 and 28 after calving, their control cows had ~5.5, 7.0 and 5.5% total fat and 3.0, 4.0 and 2.2 % TAG on wet tissue weight basis. The values for total fat and TAG obtained in current study were similar to these for the same biopsy sample days and when also expressed on wet weight basis (9.2, 10.16 and 8.45%; and 2.02, 2.58 and 1.89%).

Only one study was found that investigated the relationship of hepatic MTP gene expression (mRNA) and severity of fatty liver (TAG accumulation) in dairy cows during the transition period (Bremmer et al., 2000). They evaluated TAG accumulation, MTP mass, activity and mRNA MTP expression in liver samples collected on d -27, 2 and 35 relative to calving. The TAG accumulation at -27, +2 and +35 d from parturition was 1.8, 11.8 and 10.2% on a DM basis. Differences from prepartum levels were observed at d +2 and +35, but no differences were seen between postpartum sampling days. With regard to MTP, when levels were compared to prepartum (basal), there was no effect of sampling day on hepatic MTP mass, but there was on mRNA and activity. The mRNA expression was higher at d 2, but not at d 35, which did not differ from d 2. Enzyme activity was at its lowest at the day of calving. Based on these results, the authors indicated that MTP does not play a role in the development of fatty liver during the periparturient period of dairy cows. The relevance of MTP was questioned because, despite increase in the mRNA expression at calving, the protein mass was unchanged and activity was low on that day, even though there was a significant increase in TAG accumulation (555%).

Based on results of previous studies, it can be speculated that upregulation of MTP mRNA expression occurs in liver cells when there is need to excrete the accumulated TAG via VLDL. In fact, it has been observed that the availability of intracellular TAG seems to be critical for the activity of MTP on VLDL secretion, and it has been shown that lipids can act as transcription factors for MTP gene expression in humans and rats (White et al., 1998). Increased intracellular TAG would upregulate the MTP gene expression to cope with the demand for protein, perhaps as seen for cows in TRT II, and a decrease of TAG would down regulate mRNA expression similar to what was seen for TRT IV. The stability of MTP mass would be explained by the fact that the protein use equals its output from the endoplasmic reticulum in the bovine hepatocyte. Therefore, no increase in mass would be observed, and this would create a stable gradient of MTP protein in the cell, regardless of the fact there was accumulation of TAG or other lipids. The reason why there was no increase in MTP activity at parturition in the previous study remains to be determined, but it may be related to the necessity of the liver to retain and utilize some of this TAG to produce ketone bodies, which then would be used as an energy source by extra-hepatic tissues at a time when almost all glucose

(80%) is taken up by the mammary gland to support synthesis of milk and for mammary metabolism (Bell, 1995).

The relevance of these findings on liver function and lipid accumulation in cows that were supplemented with low doses of bST that began at calving (TRT II), is that the bST negatively impacted the secretion of TAG as VLDL by the liver at the last biopsy day. This apparently occurs despite the high MTP gene expression at that day. On the other hand, hepatocytes of cows treated during both prepartum and postpartum periods (TRT IV) were influenced by other factors that did not lead to increased NEFA mobilization, since this group of cows had the lowest mean concentrations of NEFA in plasma during the postpartum period (Table 29) and thus, there was less deposition of fat, and apparent down regulation of MTP gene expression. For cows in TRT groups I and III, despite having TAG accumulation in liver that was similar to TRT IV at d 28 postpartum (Figure 20), they had similar MTP gene expression to that of TRT II, which had the highest TAG levels in the liver at the last biopsy day (Figure 23). The reasons why these observations occurred remain to be determined but may be related to factors other than TAG regulation of mRNA MTP expression.

Another important aspect of hepatic metabolism relates to carbohydrates and how bST-supplementation affects their metabolism. This would add to greater understanding of carbohydrate metabolism than can be gotten from measurement of glucose concentrations in plasma during the transition period. To accomplish this the relative abundance of hepatic mRNA encoding two key enzymes of the gluconeogenic pathway [*pyruvate carboxylase* (PC) and *phosphoenolpyruvate caryboxykinase* (PEPCK)] were quantified. In ruminants, blood glucose used for lactose synthesis in the mammary gland is derived from two sources: diet and gluconeogenesis in liver. Because dietary carbohydrates are extensively fermented to volatile fatty acids in the rumen, less than 15% of blood glucose is derived from dietary sources. Thus, gluconeogenesis by liver provides at least 85% of glucose taken up by the mammary tissue (Bauman and Elliot, 1983).

In the biochemical pathway of gluconeogenesis, PEPCK is a crucial enzyme that converts cytosolic oxaloacetate to phosphoenolpyruvate using carbons from the gluconeogenic precursor propionate, whereas PC catalyses the ATP-dependent carboxylation of pyruvate to form oxaloacetate, and may be critical in determining use of lactate and amino acids to form pyruvate for glucose formation (Velez and Donkin, 2004). There are major differences between non-ruminants and ruminants with regard to precursors utilized in the gluconeogenic pathway. In guinea pigs and sheep (herbivores) propionate is utilized by hepatocytes to a much greater extent than in cells from rats and mice, which also do not utilize as much lactate. The differences indicate that herbivores are better adapted to utilize volatile fatty acids produced by rumen fermentation of ingested carbohydrates, especially propionate, and therefore the PC and PEPCK enzymes are of key importance in this process (Lomax et al., 1986). Therefore, it is likely that the hepatocytes will respond to the presence of these substrates by increasing the expression of genes that encode these enzymes.

Results of the current study showed that there was no effect of bST-supplementation on the mRNA expression for PC, but clear differences of biopsy day on PC expression were seen (Table 33). The expression was greatest immediately after calving (d +2) but it decreased to the lowest level observed across biopsy days on d 28

(Table 33 and Figure 21). As for all other liver measures evaluated, the change was greatest on d 28 postpartum. Cows supplemented with bST during both prepartum and postpartum periods (TRT IV) had lower PC gene expression than non-supplemented control cows (Table 35). For PEPCK, bST-supplementation impacted gene expression throughout the transition period. Across all biopsy days, cows supplemented during the both periods (TRT IV) had the lowest PEPCK expression during the period. In addition to TRT effects, there was also a day effect observed. Averaged across cows on all TRT, peak PEPCK expression occurred at d 14 postpartum, but was lowest and similar to prepartum levels in the d 28 biopsy samples. As already indicated, all liver variables evaluated showed differences due to treatment. Cows on TRT IV had the lowest PEPCK mRNA expression on d 28 postpartum (Table 35, Figure 22).

The increase in PC gene expression at d 2 postpartum, and the increase in PEPCK at d 14 in all experimental groups indicated that all cows were using increased amounts of lactate and/or AA as gluconeogenic precursors at that time. In fact, based on observations of Blum et al. (1985) and of Bell and Bauman (1997), there is extensive mobilization of endogenous protein sources during the first several weeks after parturition; this increases the AA in blood that can be used as precursors to form pyruvate. Greenfield et al. (2000) also observed an increase in PC gene expression and activity at the time of parturition in hepatocytes of dairy cows. In their study, PC mRNA expression increased at about 1 wk after calving, and then decreased to prepartum levels at 56 DIM. However, increase in abundance of PEPCK mRNA expression occurred at 28 d postpartum, much later than the increase in PC that occurred shortly after parturition. In the current study, there was an increase in PC mRNA expression before the PEPCK mRNA expression was upregulated to utilize the extra pyruvate formed from mobilized AA. Because no differences in PC mRNA expression were detected among TRT groups (Table 32), the use of AA as gluconeogenic precursors apparently was similar for all groups of cows.

Despite the similarity of PC expression, cows that were treated with bST during the prepartum (TRT III and IV) did have lower PEPCK gene expression than other groups, with the lowest abundance observed in TRT IV cows, due to the low gene expression observed in samples collected on the last biopsy day (28 DIM; Table 35). For all other biopsy days, PEPCK gene expression in this group was similar to that in all other TRT groups. It is possible that the hepatocytes at this time were preferentially using glycerol mobilized from the adipose tissue as a major gluconeogenic precursor. For this to occur, increased activity or gene expression of fructose 1,6-biphospatase (FBPase, Rukkwamsuk et al., 1999) would be seen, because this is the key enzyme in the gluconeogenic pathway that uses glycerol. This enzyme was not measured in the current study so this can not be verified. This interpretation has support, because fat and TAG deposition and MTP mRNA expression all were low in this group at that time (Table 34 and 36). This means that less TAG molecules were formed and stored in the hepatocyte and/or secreted as VLDL, despite similar concentrations of NEFA in plasma compared to cows on TRT II (Table 29); the latter cows had greater TAG deposition in liver on that day. Instead, the glycerol backbone of the TAG was used as a gluconeogenic precursor and the fatty acids were completely or partially oxidized to ketone bodies, which then were used to a greater extent by extra-hepatic tissues as a source of energy (Table 29). At the same time the mammary gland was using extensive amounts of glucose that was extracted from the available in blood to support increased lactose synthesis and mammary metabolism. Also, this improved hepatic adaptation to lactation to meet energy needs, and to spare glucose did not cause a decrease of glucose in blood (Table 29), confirming that it was an efficient metabolic adaptation. Furthermore, there was an increase in milk production by cows in this group during early lactation (3-63 DIM; Table 43).

An important question that remains to be answered is whether bST exerted a direct action in the hepatocyte that led to the pattern of gene expression of these enzymes during this critical period, or whether the results seen occur because of the nutrient partitioning seen during the transition period. At present, this question can not be answered. The only two studies published (Pershing et al., 2002; Velez and Donkin, 2004) that also quantified gene expression of these key enzymes in dairy cows that were supplemented with bST, were conducted using cows in which lactation was already established and the results are contradictory. In the study completed by Pershing et al. (2002), mRNA abundance of PC, PEPCK and MTP was quantified 7d after they were supplemented with the full dose of bST (500 mg of bST/14 d). They used multiparous Holstein cows when they were 80 DIM. No differences in mRNA expression were observed between control or bST-supplemented cows for any of the enzymes.

However, in the study of Velez and Donkin (2004), supplementing multiparous Holstein cows with 3 bi-weekly injections of 500 mg of bST at 116 DIM increased PEPCK mRNA expression during the 3rd cycle of bST-supplementation. There was both an increase in abundance, as well as a tendency for the *in vitro* mRNA elongation in nucleus of cells of cows that were supplemented with bST. Their interpretation was that the increase in DMI that occurs following the MY increase in bST-supplemented cows during established lactation leads to the increase in gluconeogenic activity as demonstrated by Knapp et al. (1992), and this increase in activity drives the upregulation of the PEPCK gene expression. Differences in results of the two studies relative to apparent effects of bST-supplementation likely arises, in part, because in the study of Pershing et al. (2002) only one supplementation cycle was used before making quantitative measures of the mRNA for the enzymes and this probably was insufficient time and/or stimulation to allow metabolic adaptations to occur. What was not determined in the most recent study by Velez and Donkin (2004) was whether the increase in transcription of the PEPCK gene was due to the direct action of somatotropin on liver, whether there was modulation of the actions by other hormones and metabolites, or whether both of these precursors occurred.

An important goal of this study was to determine if supplementation of low-doses of bST during the transition period and/or early lactation would favor greater milk yield of dairy cows without changing milk composition and without negative effects on their health or conversely whether improved health due to bST supplementation during the transition period favors greater milk yield. The health information obtained for all experimental cows across groups during the first 60 DIM indicates that incidence rates for all diseases were similar among all treatment groups and cohorts. These results, even though from a small group of supplemented cows and cohorts, agree with those of a number of published studies that were summarized by Kelton et al. (1998).

Among all TRT groups in the current study, the incidence rate of retained fetal membranes (RFM), metritis (MET), mastitis (MAST) and digestive problems (DIG) were above 10% (high incidence, Table 39). The range of incidence rates observed in the current study and reported in the literature were: for RFM they ranged from 0.0 to 25.5% and 1.3 to 39.2%; for MET, they ranged from 32 to 42.3%, and 2.2 to 37.3%; for MAST, values ranged from 14.8 to 30.7%, and 1.7 to 54.6%; and for DIG, values in current study ranged from 0.0 to 19.2% (Table 39).

No values for DIG, as used in the current study, are found in the literature because it is a general term for a group of diseases characterized by dysfunction of the reticulorumen. These dysfunctions can be primary, where the reticulorumen is directly affected and responsible for the major disease signs, or secondary, which are the sequelae of systemic problems or disease in other organ systems. Examples of primary indigestions are reticuloruminal motor disorders/diseases of the rumen wall (hardware disease, frothy bloat, free gas bloat, reticulitis/rumenitis, rumenal parakeratosis, vagal indigestion, obstruction of the cardia, obstruction of the reticuloomasal orifice and diaphragmatic hernia); and reticuloruminal fermentative (inactive rumen microbial flora, simple indigestion, acute rumen lactic acidosis, chronic rumen acidosis and rumen alkalosis). Examples of secondary indigestions are secondary reticuloruminal motor inactivity and secondary reticuloruminal miclofora inactivity (Garry, 1996). Therefore, experimental cows with a case of indigestion/digestive problem could have had one or more of the diseases of the reticulorumen.

The incidence rates of ketosis (KETO), milk fever (MF), left/right displacement of the abomasum (L/R DA) and lameness (LAME) were less 10%, and similar among TRT groups. These agree with literature numbers (Table 39). This indicates that bST-supplementation in low-doses did not lead to ketosis, as observed by Eppard et al. (1996) or Laven and Andrews (1998), where the full dose of bST, as used to enhance lactation, was supplemented to cows during the transition period. In those studies, NEFA and B-HBA concentrations were greater in supplemented groups of cows. These results differ from that seen in the current study, as discussed previously.

For MF, the anionic diet fed to all cows was effective in preventing the disorder. Range of incidence appearing in the literature is from 0.03 to 22.3%; whereas incidence of cows and cohorts in current study was from 0 (TRT II, III and IV and cohorts) to 7.7% (controls), well below average values reported. For L/R DA, the incidence rate observed ranged from 3.7 to 8.0% and was slightly greater than the reported range of 0.3 to 6.3%, but included only LDA, whereas in current study the range presented included both LDA and RDA so slightly greater incidence may be expected. Finally, for lameness (LAME), the incidence rates ranged from 3.7 to 6.3%, also in the range of values reported in the literature, which were from 1.8 to 30% (Kelton et al., 1998).

Overall, these results indicate that the use of low doses of bST did not adversely affect health of dairy cows during the transition period/early lactation compared to non-supplemented controls and cohorts. Indeed, it seems that cows of TRT group IV had reduced incidences of RFM and DIG, and there was a tendency for this group to have a greater number of cows that did not have a case of any disease, so if anything, they may have been more healthy than the non-supplemented groups of cows.

As is known, some diseases/disturbances are predisposing factors for other diseases (Table 40; Melendez et al., 2003). In the current study, cows that had RFM were more prone to develop MET; cows that had ketosis tended to develop more digestive problems; and there also was a strong association between calving season and digestive problems. Cows that calved during season II (November-February) were more likely to have a digestive problem than cows calving during season I. Melendez et al. (2003) observed that cows with RFM were 37.3-times more likely to develop MET than those that did not have the disease. In addition, cows with dystocia were 2.7-times more likely to develop RFM than healthy cows. In the current study, no significant association between dystocia and RFM was seen, perhaps because there were only 25 cows per group, whereas the average number of cows per TRT group in the previous study was 160. In addition, the incidence of dystocia was higher than expected, which they interpreted to mean there was very aggressive calving intervention by the farm crew. In fact, despite the significant increase in calving difficulties observed with cows in the control group (Table 37 and 38), the increase was not severe enough and did not impact the occurrence of RFM (Table 40), indicating that calving intervention was not aggressive.

The reason for a significant decrease in incidence of RFM in the group of cows treated during both prepartum and postpartum periods (TRT IV) is difficult to explain. RFM is a complex disease associated with several predisposing factors, mostly evident before or at parturition. In addition, RFM is positively related to the duration of pregnancy, which influences subsequent prophylactic and therapeutic measures. However, numerous additional factors (nutritional, hormonal, stress and hereditary) that prevent the detachment of the placentomes, which results in RFM, must be considered under physiological, as well as, pathologic conditions of the gestation period (Grunert, 1986). Because the mechanism by which low doses of bST appears to improve the detachment of the placentomes is not obvious and no data were collected to aid in developing a logical explanation, all that can be mentioned is that the results are preliminary and present the interesting possibility that bST-supplementation may reduce the occurrence of this costly disease.

For digestive problems, cows on TRT IV also were less likely to experience a problem of indigestion, whereas cows that calved during the cooler season were more likely to develop a digestive problem. It seems that the greatest disturbances related to indigestion were reticuloruminal fermentative which included inactive rumen microbial flora, simple indigestion, acute rumen lactic acidosis, chronic rumen acidosis, and rumen alkalosis. This interpretation is based upon the fact that no specific diagnosis of a disorder was made that required surgical intervention. These fermentative dysfunctions are related to a rumen environment not adapted to changes in feeds that normally occur following calving, or to a sudden high intake of feeds high in concentrate content in a cow with a non-adapted rumen (Garry, 1996). Because feed intake was not measured in the current study, it is only possible to speculate relative to the cows in this study. Perhaps cows on TRT IV were better adapted to the change in diet due to a smaller decrease in feed intake prepartum than seen in the other groups of cows, and this allowed better maintenance of activity of the microbial population in the rumen (Goff and Horst, 1997). On the other hand, cows that calved during season II likely had an increased feed intake of a diet high in energy content (concentrate, fermentable carbohydrates, Table 1) due to the higher energy requirement for body maintenance during cooler months of the year (National Research Council [NRC], 2001). Perhaps this scenario caused simple indigestions or mild acidosis in these dairy cows.

No differences in variables that were developed to describe reproductive performance were observed .There was only a trend for improved pregnancy rates of cows supplemented with low doses of bST during postpartum (Table 40); all other variables were similar across treatments (Tables 39 and 40). This indicates that bST supplemented during the transition period does not negatively impact reproductive performance of dairy cows during lactation. In addition, results are similar to Melendez et al. (2003), and therefore likely are valid, although cow numbers are small. The reproductive performance of dairy cows has been extensively studied past decades (Lucy et al., 2001), and a thorough discussion of this topic it is not an objective of this study.

An important issue that needs to be considered is whether the metabolic changes described have a direct impact on the physiology of mammary tissue, particularly during the prepartum period, which is a time period during which it may be sensitive to low doses of supplemented bST. Indeed, several hormones showed increases in concentrations in plasma of the supplemented cows during the prepartum period (somatotropin and insulin-like growth factor-I), and they also play an important role in decreasing *in vitro* rate of apoptosis of mammary epithelial cells. It is possible that they can improve cell survival (Accorsi et al., 2002), and as consequence impact milk production positively during the subsequent lactation. In their study, Accorsi et al. (2002) evaluated mammary tissue collected from Italian-Friesian cows during late lactation (7-10 mo). Tissue was treated with a group of hormones made up of hydrocortisone, 17 β-estradiol, progesterone, insulin, ST, IGF-I and PRL, or with different groups of hormones that were missing one of them. They observed that treatment of mammary tissue with the full group of hormones suppressed cellular apoptosis, whereas the absence of a single hormone or growth factor (PRL, GH or IGF-I) caused an increase in apoptotic DNA fragmentation after 24 h in culture. In addition, expression of IGF binding protein-5

(IGFBP-5), one of the binding proteins that regulates IGF-I biological actions (Jones and Clemmons, 1995), was at it lowest when cells were treated with the complete hormone media, and there also was a very low rate of apoptosis. When cells were cultured without PRL or ST or IGF-I, gene expression of IGFBP-5 was greater than when cells were exposed to the hormonal mix that included all the hormones. Implications are that during late lactation, a plethora of hormones and IGF-I are required for survival of mammary epithelial cells, in that, expression of IGFBP-5 decreases, which, in turn, causes an increase in free IGF-I in the media/tissue that exerts its biological actions, which includes cell survival.

Despite evidence that IGFBP-5 plays a role in mammary epithelial cell survival during late lactation in ruminants, does it have the same activity during the dry period? Evidence in the literature indicates that IGFBP-5 has a role in inducing mammary cell apoptosis during the dry period in mice, but in ruminants IGFBP-3 seems to play a greater role. This binding protein is the major IGFBP in bovine mammary cells and in milk, and its major function is to obstruct IGF biological action. Blood concentrations of IGFBP-3 are greater during both the prepartum period and during involution after completion of the lactation than during any other phase of the lactation cycle (Baumrucker and Erondu, 2000; Flint et al., 2001; Blum and Baumrucker, 2002). In addition, it has been observed that treatment of 18 mo old dairy heifers with exogenous somatotropin ST causes proliferation of mammary epithelial cells by increasing IGF-I concentrations in plasma, and this is accompanied by a decrease in concentrations of IGFBP-3 in the mammary tissue (Berry et al., 2001).

Although no direct measures of the mammary epithelial cell population or survival were attempted during the current experiment, it is plausible that in groups of cows that were supplemented prepartum, the small, but positive increase in IGF-I concentrations observed may have positively impacted the survival or renewal of mammary epithelial cells (Capuco et al., 1997), despite the increase in prepartum concentrations of IGFBP-3 in plasma. Because an increase in numbers of mammary epithelial cells is directly associated milk production in cows (Akers, 2000), this would be reflected by an increase in milk production observed in TRT groups of cows supplemented with bST prepartum compared to non-supplemented controls (Table 43). In addition, postpartum supplementation of bST, which also increases plasma concentrations of IGF-I in plasma during the postpartum period, which is necessary to maintain the survival of mammary epithelial cells during the early stages of lactation, may have an additive effect on the milk production and lactation persistency in TRT IV cows (Forsyth, 1996; Stefanon et al., 2002). This effect could be manifested by a significant increase in milk yield during lactation.

Based upon MY, BW and BCS trends seen in previous studies and the current study, we speculate that the DMI intake of experimental cows increased during early lactation (0-63 DIM). Overall, cows on TRT IV that were supplemented with bST during both prepartum and postpartum periods showed greatest increase in milk yield compared to control cows (~20%, 34.81 vs. 41.3 kg/d) during the early lactation period (3-63 DIM, Table 43). The BW and BCS data show that postpartum losses in BW and BCS were similar across treatment groups and, in fact, did not differ (Tables 17, 18 and 19). Therefore, to increase their milk production, while at the same time experience no loss of body condition or BW, they must have increased DMI and/or efficiency of milk production to meet their needs for the extra nutrients for body maintenance, metabolism and milk production. In fact, we speculate that they did both of these things; increased DMI and efficiency, as in other studies (Gulay et al., 2003b).

Based on information contained in the 2001 edition of National Research Council (NRC, 2001), dairy cows that weigh 680 kg (large breeds such as Holsteins) at the beginning of the lactation (estimate at 11 d in milk), require 15.1 kg of DM of a diet containing a total of 32.8 Mcal of NE_L (2.17 Mcal NE_L/kg DM) and 18.3 % CP to produce an average of 30 kg/d of milk with 4% milk fat and 3% milk protein. For this same cow to produce 40 kg/d of milk with the same concentrations of nutrients, requirements will increase to 17.4 kg of DM of a diet containing 40.2 Mcal of NE_L (2.31 Mcal of NE_L) and 19.8% CP. These calculations indicate that dairy cows require 1-1.09 Mcal of NE_L for each kg of milk produced during early lactation.

Because all experimental cows were consuming the same TMR that contained 1.60 Mcal NE_L/kg DM with 17.45 % CP (Table 1), controls and TRT IV cows must have consumed, respectively, an average of 20.5 and about 25.0 kg of DM daily. This represents a 20-25% increase in feed consumption to match the requirements for production. In fact, this is what Garcia-Gavidia (1998) observed when DMI was measured. In his study, multiparous Holstein cows supplemented with 5 mg of bST/d beginning at ~3 wk before parturition through 56 DIM had about 17% greater DMI than control cows during the first 65 DIM. This was associated with a numerical increase of 1.58 kg/d of total milk produced in the supplemented cows. However, in the study by Gulay et al. (2004) no differences were detected with regard to changes in DMI in cows supplemented with 10.2 mg/d of bST during the early postpartum period. However, their postpartum measurements of feed intake were only through 28 DIM, and therefore did not account for significant increases in DMI that likely occurred beyond 28 DIM.

Conclusions

Supplementation of dairy cows with low doses of bST (10.2 mg/d; 166.7 mg/14d of POSILAC®, Monsanto Company, St. Louis, MO) during the transition period and early lactation was effective in improving the metabolic adaptations during this critical phase of the lactation cycle. Evidence was obtained to indicate that prepartum and postpartum supplementation of low doses of bST optimized changes in metabolic adaptations of the dairy cow that favored greater milk production. The prepartum supplementation alone did not provoke beneficial effects on subsequent milk production, although specific changes in the physiology of the animal were evident. When cows were supplemented only during the postpartum period, fatty acid mobilization from adipose tissue and increased triacylglycerol accumulation in liver led to increased B-HBA. Moreover ,it did not cause a positive impact on milk production compared to controls, although milk production was numerically greater.

The increase in milk yield of cows treated during the prepartum, postpartum and early lactation periods was about 20% greater than controls due to the increased stimulation of mammary gland synthetic capacity, and because of improved metabolic adaptations to support the milk synthesis. Maintenance of BCS and BW indicate that DMI was increased, and levels of minerals and metabolites in plasma [calcium (Ca), glucose, non-esterified fatty acids (NEFA) and β-hydroxybutyrate (B-HBA)] was similar to or better than control during the transition period.

Liver metabolism of lipids and carbohydrates of treated cows was similar or better than control cows during the transition period. There was no greater accumulation of total fat or triacylglycerol (TAG) in liver, and the gene expression of key enzymes of the metabolism of fat and carbohydrates did correspond to the improved metabolic status. Because of the likely decrease in GHR 1A in liver during the transition period, the actions of bST on the modulation of gene expression of liver enzymes seems to occur as secondary response to coordinated changes in hormones (ST and INS), growth factors (IGF-I) and intermediary metabolites (glucose, NEFA and B-HBA) that were shown to change when dairy cows were treated with low doses of bST during the transition period.

Importantly, there was no evidence that bST-supplementation of low doses increases the incidence of common diseases of the transition period and early lactation. In fact, supplementation of bST tended to promote better health of dairy cows when it was supplemented both prepartum and postpartum. Whether the improvement in milk production results, in part, because of better health remains unanswered. Also, there is no impairment in the reproductive performance of supplemented cows during the lactation period, indicating that there are no deleterious delayed effects of bST on reproduction.

Although no economic measures were included in the data collected during this study, it is possible to do a retrospective analysis and evaluation of probable costs and benefits. Based on the Dairy Business Analysis Project of the University of Florida (DBAP, de Vries et al., 2003), total dairy farm income is based mainly on the price of milk sold; it comprises about 90-95% of the total income of the commercial dairy farmer in the State of Florida. Therefore, to increase income of a dairy farm more milk must be produced. The costs of production, which includes cost of personnel, feed, machinery, livestock replacement, milk marketing, depreciation and others, represent about 70-80% of the price of milk.

In the current study, cows supplemented with bST during the prepartum, postpartum and early lactation period (TRT IV) produced 682 kg more milk during the first 150 DIM. Accounting for the cost of bST supplementation (about $ 2.66 per dose and a total of $ 18.62 for the 7 doses used during the time period before full dose of bST was started at about 60 DIM), the costs of production and today's price of milk paid to the dairy farmer (~$ 20.00/45.5 kg), there would be a profit of $ 87.70 per cow supplemented. In addition, the prevention of incidences of retained fetal membranes (5 cases, $ 285.00 per case) and digestive problems (5 cases, ~$ 100.00 per case), will account for an additional $ 1,900.00 of income, or decrease in expenses for the farm. Therefore, for the group IV cows (n=25) there is added income (MY and health care) of $ 9,445.00 to the farm, with a profit of $ 4,092.50. For a group of 100 cows, the increase in profit would be of $ 16,370.00.

In the State of Florida, based upon the latest estimates of cow numbers, there are ~150,000 lactating cows (FASS, 2004). From this total, 65% are assumed to be multiparous cows that will qualify for this practice (97,500), and we estimate that there will be a 60% adoption of the supplementation procedure described. Based upon these estimates, about ~58,500 cows likely will be supplemented with low doses of bST during the prepartum, postpartum and early lactation periods, and they will benefit from this management practice. Therefore, the adoption of this management practice by state of Florida dairy producers will add about $ 957,645.00, or ~ close to $ 1 million per lactation.

Besides the undeniable economic benefits, it is equally important that this management practice could be implemented by the dairy industry to improve the well-being of their dairy cows, because it has the potential to reduce incidences of common transition cow diseases, as well as improve milk production. This ultimately would benefit the dairy industry in our region and throughout the USA, leading to decreased numbers of cows at the farm level to achieve the desired total milk production. This would be expected to decrease the environmental impact of intensive dairying. Therefore, this very small investment could bring enormous benefits to dairy cow health and milk production, increased income, which would translate into better and less expensive products being available to the consumer.

APPENDIX A
RESIDUAL CORRELATION COEFFICIENTS AMONG HORMONES AND METABOLITES MEASURED FROM 21 D PREPARTUM THROUGH CALVING IN PLASMA OF HOLSTEIN COWS

	Variables[1]						
	ST	IGF-I	Ca	GLC	NEFA	B-HBA	BW
INS	-0.096**	-0.002	-0.058	0.127**	-0.156**	-0.025	
ST		-0.035	-0.055**	-0.020	0.145**	0.054	
IGF-I			0.208**	-0.013	-0.262**	-0.193**	
Ca				-0.017	-0.134**	-0.066*	
GLC					-0.034	-0.031	
NEFA						0.298**	
BCS[3]							0.064

[1] Insulin (INS), somatotropin (ST), insulin-like growth factor-I (IGF-I), calcium (Ca), glucose (GLC), non-esterified fatty acids (NEFA), β-hydroxybutyrate (B-HBA), body weight (BW) and body condition score (BCS). † *P*≤0.10; * *P*≤0.05;** *P*≤0.01. Mathematical model used: bST, season, bST*season, using cow (bST*season) as error term, with day to the third order as covariate.

APPENDIX B

RESIDUAL CORRELATION COEFFICIENTS AMONG HORMONES AND METABOLITES MEASURED DURING THE EARLY POSTPARTUM PERIOD (0-63 DIM) IN PLASMA OF HOLSTEIN COWS

	Variables[1]						
	ST^2	IGF-I^2	Ca^3	GLC^3	NEFA^3	B-HBA^3	BW^2
INS^2	0.023	0.135**	0.037	0.132**	-0.170**	-0.149**	
ST		0.142**	0.048	0.043**	-0.033	0.047	
IGF-I			0.017	0.119**	-0.211**	-0.235**	
Ca				-0.002	-0.113**	-0.085**	
GLC					-0.116**	-0.356**	
NEFA						0.291**	
BCS^3							0.502**

[1] Insulin (INS), somatotropin (ST), insulin-like growth factor-I (IGF-I), calcium (Ca), glucose (GLC), non-esterified fatty acids (NEFA), β-hydroxybutyrate (B-HBA), body weight (BW) and body condition score (BCS). [2] From 0-63DIM. [3] From 0-28 DIM. † $P \leq 0.10$; * $P \leq 0.05$;** $P \leq 0.01$. Mathematical model used: bST, season, bST*season, using cow (bST*season) as error term, with day to the third order as covariate.

APPENDIX C
SUMMARY OF HETEROGENEITY OF REGRESSION TESTS FOR VARIABLES EVALUATED DURING THE EXPERIMENT

Effect[1]	df	MS Het.	F. Het	P
Treatment (MY)	9	1026.55	20.77	0.0001
Treatment (BW)	9	3415.23	3.14	0.05
Treatment (BCS)	9	0.14	2.43	NS
Treatment (ST)	9	612.74	38.08	0.0001
Treatment (IGF-I)	9	6761.14	4.50	0.01
Treatment (B-HBA)	9	266.14	7.14	0.0001

[1] MY (milk yield), BW (body weight), BCS (body condition score), ST (somatotropin), IGF-I (insulin-like growth factor-I) and B-HBA (β-hydroxybutryrate).

APPENDIX D
RESIDUAL CORRELATION COEFFICIENTS AMONG LIVER MEASURES DURING THE TRANSITION PERIOD OF HOLSTEIN COWS

	Variables[1]			
	TAG	PC	PEPCK	MTP
LFAT	0.923**	-0.012	-0.227†	-0.172
TAG		-0.084	-0.300**	-0.198
PC			0.278*	0.224*
PEPCK				0.293*

[1] Percentage of fat in liver on wet basis (LFAT), percentage of triacylglycerol in liver on wet basis (TAG),enzymes *pyruvate carboxylase* (PC), *phosphoenolpyruvate carboxykinase* (PEPCK) and *microsomal triacylglycerol transfer protein* (MTP). † $P \leq 0.10$; * $P \leq 0.05$;** $P \leq 0.01$. Mathematical model used: bST, season, bST*season, using cow (bST*season) as error term, with day to the third order as covariate.

APPENDIX E

SUMMARY OF TREATMENT EFFECTS OF MULTIPAROUS HOLSTEIN COWS SUPPLEMENTED WITH bST DURING PREPARTUM OR DURING POSTPARTUM PERIODS COMPARED TO NON-SUPPLEMENTED CONTROLS

	Variable					
	Milk Yield[1]	BW/BCS[2]	Hormones[3]	Metabolites[4]	Liver Function[5]	Health[6]
Treatment						
bST Post[7]	Similar to control group.	Absolute values and changes during experiment were similar to control group.	Higher mean concentration of somatotropin than control group during postpartum period.	Higher mean concentration of B-HBA than control group during the postpartum period.	Higher TAG deposition and higher PC and PEPCK mRNA abundance than control group at 28 DIM.	Incidence of diseases was similar to control group.
bST Pre[8]	Similar to control group.	Absolute values and changes during experiment were similar to control group.	Absolute values and changes during experiment were similar to control group.	Absolute values and changes during experiment were similar to control group.	Absolute values and changes during experiment were similar to control group.	Incidence of diseases was similar to control group.

[1] Measured 3x/d. [2] Measured weekly from 21 d prepartum through 63 DIM, and bi-weekly from 64-150 DIM. [3,4] Concentrations of insulin, somatotropin, insulin-like growth factor-I, calcium, glucose, non-esterified fatty acids and β-hydroxybutyrate in plasma obtained 3x/wk from 21 d prepartum through 28 and 63 DIM.

[5] Total fat and triacylglycerol (TAG) deposition, and steady state mRNA abundance of *pyruvate carboxylase* (PC), *phosphoenolpyruvate carboxykinase* (PEPCK) and *microsomal triacylglycerol transfer protein* (MTP) in liver samples obtained at -21, +2, +14 and +28 d from calving in a subset of ~9 cows per treatment group. [6] From retrospective data collected from 0-63 DIM. [7] n=25; supplementation with 10.2 mg/d of bST began at about 24 h following calving and extended through 63 DIM. [8] n=27; supplementation with 10.2 mg/d of bST began at 21 d prepartum and extended through calving.

APPENDIX F
SUMMARY OF TREATMENT EFFECTS OF MULTIPAROUS HOLSTEIN COWS SUPPLEMENTED WITH bST DURING PREPARTUM AND POSTPARTUM PERIODS COMPARED TO NON-SUPPLEMENTED CONTROLS

	Variable					
	Milk Yield[1]	BW/BCS[2]	Hormones[3]	Metabolites[4]	Liver Function[5]	Health[6]
Treatment						
bST Pre/Post[7]	Cows produced 12.8% more milk daily than control group during the first 150 DIM.	Absolute values and changes during experiment were similar to control group.	Higher mean concentration of somatotropin and IGF-I than control group during the whole experiment.	Absolute values and changes during experiment were similar to control group.	Similar TAG deposition, and decreased PC and MTP mRNA abundance than control group at 28 DIM.	Cows tended to be were healthier than controls, and were less likely to have retained fetal membranes and digestive problems.

[1] Measured 3x/d. [2] Measured weekly from 21 d prepartum through 63 DIM, and bi-weekly from 64-150 DIM. [3,4] Concentrations of insulin, somatotropin, insulin-like growth factor-I, calcium, glucose, non-esterified fatty acids and β-hydroxybutyrate in plasma obtained 3x/wk from 21 d prepartum through 28 and 63 DIM. [5] Total fat and triacylglycerol (TAG) deposition, and steady state mRNA abundance of *pyruvate carboxylase* (PC), *phosphoenolpyruvate carboxykinase* (PEPCK) and *microsomal triacylglycerol transfer protein* (MTP) in liver samples obtained at -21, +2, +14 and +28 d from calving in a subset of ~9 cows per treatment group. [6] From retrospective data collected from 0-63 DIM. [7] n=25; supplementation with 10.2 mg/d of bST began at 21 d prepartum and extended through 63 DIM.

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BIOGRAPHICAL SKETCH

Márcio Liboni is a native Brazilian born November 5, 1974, in and raised in Londrina, Paraná. His primary, secondary and high school education was undertaken at the Maxi School in Londrina from 1981 until 1991. In the following year (1992) he began his college education at the College of Veterinary Medicine, State University of Londrina. During his senior year, he received professional training through an internship at Lander Veterinary Clinic, Turlock, California, from August to October, 1996. Following his graduation as a Médico Veterinário from college in December, 1996, he was accepted into the Large Animal Clinic, Surgery and Reproduction Residency Program of the State University of Londrina. In February, 1999, he graduated from the Residency Program and at the same time was awarded a scholarship from the Rotary Foundation to enroll in the Master of Science program at the Department of Animal Sciences of the University of Florida. In May, 1999, he received supplemental funds from the Department of Dairy Sciences to undertake the graduate program, and in August 1999, he began his graduate studies. In August 2001 he was awarded with the Master of Science Degree from the University of Florida, and at the same time he began his Ph.D. studies after receiving an assistantship from the Department of Animal Sciences of the University of Florida. On March 8, 2002, he married Kellym Candotti, and they came to Gainesville, Florida to begin a new life and family and to continue his graduate studies. He will receive his Ph.D. degree during summer commencement, 2004, after which he will return to Brazil.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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